Physiological Stress, Smoltification and Seawater Adaptation in New Zealand's Sockeye and Quinnat Salmon.

A thesis
submitted in fulfilment
of the requirements for the Degree
of

Doctor of Philosophy in Zoology
in the
University of Canterbury

by

Craig E. Franklin.

University of Canterbury

1989

Dedicated in loving memory to my father

Leonard Edward Franklin

(1917-1988).

TABLE OF CONTENTS

Abstract.

This study investigated smoltification and seawater adaptability of sockeye (*Oncorhynchus nerka*) and quinnat salmon (*O. tshawytscha*). Both species were introduced into New Zealand with an anadromous population of quinnat and a totally freshwater population of sockeye salmon becoming established. The sockeye were found to be capable of successfully adapting to sea water and appeared not to have lost their hypoosmoregulatory ability despite being landlocked for approximately 90 years.

The physiological changes associated with smoltification and seawater adaptation were studied and were viewed in part with reference to physiological stress.

Salmon exposed to a handling or temperature stressor showed an increase in plasma cortisol concentrations and depending on the severity of the stressor, a change in haematocrit and an osmotic imbalance could also result.

The physiological state of the salmon (pre-smoltified, smoltified, desmoltified) was assessed in a variety of ways and the reliability of the parameters measured related to the seawater adaptability of the salmon. Increased gill Na^+-K^+-ATPase activity provided the best indicator of smoltification. Plasma cortisol concentrations also increased in smoltifying salmon.

The seawater adaptability of the salmon was found to be dependent on both the degree of smoltification that had occurred in the salmon and on the temperature of the sea water. High seawater temperatures limited the ability of the salmon to successfully adapt. Rapid increases in plasma cortisol occurred when salmon were transferred directly to sea water, but returned to basal levels within 12 hours in the fish that successfully adapted. Plasma cortisol concentrations remained elevated in the salmon that failed to adapt to the sea water.

Morphological changes in the gills of sockeye salmon that successfully and unsuccessfully adapted to sea water were also investigated.

CHAPTER 1

General Introduction

New Zealand Salmon.

Atlantic and Pacific salmon are not native to New Zealand, but rather are endogenous to the cold temperate regions of the Northern Hemisphere. Three species of salmon were introduced into the South Island of New Zealand during the mid- to late 1800's and the early 1900's. The three species of salmon that can be found in New Zealand are: Atlantic salmon, *Salmo salar* (Linnaeus), found in the Lake Te Anau - Manapouri system; sockeye salmon, *Oncorhynchus nerka* (Walbaum), found in the upper Waitaki River system; and quinnat (chinook or king) salmon, *Oncorhynchus tshawytscha* (Walbaum), the most widely distributed salmon in New Zealand, occurring primarily on the east coast of the South Island, from the Waiau River in the north to the Clutha River in the south. Salmon were specifically introduced to establish an industry and a sport fishery as the rivers of New Zealand had very few species of large fish (Flain, 1981).

Atlantic salmon was the first species to be introduced into New Zealand in 1868, all importations of ova coming from anadromous stocks from a variety of sources in the United Kingdom, Europe and Canada (Stewart, 1980; Flain, 1981). No sea run population became established but a small landlocked population remains today in Lake Te Anau.

The first attempts in 1875-1880 to introduce quinnat salmon were unsuccessful; nevertheless, between 1901-1907 successful importations of ova were made. These stocks of anadromous quinnat came from Baird Station situated on the McCloud River, a tributary of the Sacramento River in California (Flain, 1981). A sea run population was first established on the Waitaki River and other surrounding rivers were subsequently stocked from the offspring of these salmon. Quinnat salmon are the only species in New Zealand that are anadromous, migrating to the sea as part of their life cycle, although self-perpetuating freshwater populations also exist.

Sockeye salmon was the last species of salmon to be introduced into New Zealand, ova being shipped from Canada in 1901 and in 1903. Approximately

160 000 eggs were hatched and reared at a fish hatchery on the Hakataramea River, some being released into various parts of the Waitaki system, and the remainder retained in the hatchery for sea release. The sockeye introduced into New Zealand originated from a single source, a population from the Shuswap Lake, on the Fraser River in British Columbia (Flain, 1981; Graynoth and Hawke, 1983). The ova were derived from an anadromous population of sockeye, but like the Atlantic salmon, the resulting New Zealand stock became voluntarily lake limited. No sea going population has ever become established.

There is some confusion as to the origin and form of sockeye salmon in New Zealand. There are 3 recognised forms of sockeye in North America: the sockeye (the anadromous form); the kokanee (non-anadromous form which is self-perpetuating); and the residuals (the non-anadromous offspring of sea going sockeye). New Zealand sockeye appear to be comparable with the North American kokanee; however, they do not assume the bright red spawning colouration of the kokanee and instead have a spawning colouration more characteristic of residual sockeye salmon (Ricker, 1940). The North American residual sockeye represent only a small percentage of the total anadromous breeding population, whereas the New Zealand sockeye is a large freshwater breeding population and are the product of many generations in freshwater. Hardy (1983) suggests that it can not be absolutely verified that original stocks of sockeye salmon were anadromous. Some ova may have been derived from landlocked sockeye or kokanee, although most evidence points towards an anadromous stock. Nevertheless, New Zealand sockeye salmon appear to have characteristics different from the North American populations.

The Life Histories of Quinnat and Sockeye Salmon.

In North America, quinnat salmon display a spectrum of migratory patterns (Healey, 1980; Clarke and Shelbourn, 1985). Most quinnat enter the river systems and migrate upstream in September to spawn in October, these salmon being known as the fall chinook. A few quinnat (spring chinook) enter rivers between February and May to spawn in late September to October (Carl and Healey, 1984). The juveniles follow one of three types of outmigration to the ocean. Most leave soon after emergence as fry to migrate to the estuary or river mouth where they stay until they reach a size of approximately 4 g (fork length = 70mm). Other quinnat migrate after about 3 months residence in fresh water and the remaining quinnat leave the river system as yearlings (Carl and Healey, 1984).

In New Zealand, most of the information on the life history of quinnat salmon has been obtained from studies on the quinnat that spawn in the Glenariffe stream, a tributary of the Rakaia river. Adult quinnat enter the Glenariffe stream to spawn from March to June (the austral autumn to early winter) with a peak in April (Hopkins, 1981). Fry emerge from the redds between July and November and between 90 to 98% of the newly emerged juveniles (fork length = 30-40mm) migrate downstream from August to October with usually a peak in mid September (Unwin, 1981). The remaining juveniles leave the Glenariffe stream in decreasing numbers over the summer period, although in some brood years, a significant number of quinnat migrate from the stream as yearlings (12-15 months after emergence).

In North America, sockeye spawning occurs in late summer and autumn (August to November). Fry emerge from the redds in early spring (April and May) and then migrate into the adjacent lake. The juveniles are resident in the lake for 1, 2 or 3 years before migrating down to the ocean between April and June. Sockeye remain in the ocean for 1 to 4 years before returning to their natal stream.

New Zealand landlocked sockeye salmon spawn in streams entering Lake Ohau in late February and March and fry emerge three to four months later then migrate downstream into the lake (Graynoth and Hawke, 1983). In some sockeye, a further downstream migration occurs about a year later, fish travelling from Lake Ohau to Lake Benmore. Adult sockeye return to spawn at 2-5 years of age.

Anadromous salmon must be able to survive movement from fresh water to the sea and then back to fresh water.

Osmoregulation in Teleosts.

Teleost fish maintain the ionic concentrations of their body fluids at levels that are distinctly different from their freshwater or marine environments. Teleosts are provided with three main osmoregulatory structures; the gills, the intestine, and the kidney and bladder which exchange water and ions with the external environment. Of these three structures, the teleost gill plays a central role and is possibly the most important. This becomes apparent when it is realised that the gill epithelium not only constitutes more than 70% of the total body surface (Ogasawara and Hirano, 1984), but it is also the major site of the chloride cells which are intimately involved with the active uptake or excretion of monovalent ions.

In the marine environment, teleost fish have plasma osmolarities that are lower than the external environment so there is a continual loss of water by osmosis and an influx of ions by passive diffusion following the ion concentration gradients. A fish compensates for the water loss by drinking seawater. However, as it drinks it also takes in a surplus of ions, so to gain osmotically free water it must excrete this excess. Monovalent ions are eliminated chiefly by active transport across the epithelium of the gills and divalent ions are removed and excreted by the intestine and kidney (Maetz, 1971; Fontaine, 1975; Foskett *et al.*, 1983).

Freshwater teleosts have plasma osmolarities that are higher than the external environment, hence there is an influx of water (mainly through the gills) and an outflux of ions. The kidney prevents hydration of the fish by producing copious amounts of urine that is hypoosmotic to the plasma. The loss of sodium and chloride ions by the kidneys and the passive loss of these ions at the gills is countered by the active uptake of sodium and chloride across the gills (Maetz, 1971; Foskett *et al.*, 1983).

Many of the processes involved in osmoregulation are partly or completely under the control of the endocrine system. Hormones released from endocrine glands can have either direct or secondary effects on the water/ion exchange surfaces (gills, kidney etc). Hormones thought to be involved in the regulation of ions and water balance include the thyroid hormones, corticosteroids (cortisol being the principal corticosteroid in teleost fish, Idler and Truscott, 1972), prolactin, adrenaline, growth hormone and neurohypophysial peptides (Barron, 1986; Richmann and Zaugg, 1987).

Much of the work on osmoregulation in fish has concentrated on the species that exhibit diadromy and migrate (at well defined stages in their life cycle) from salt to fresh water and vice versa (see Dadswell *et al.*, 1987). These species are of special interest in the field of osmoregulation because they have to either tolerate the new salinity, or undergo a profound modification of their physiological mechanisms of iono- and osmoregulation when migrating to a completely different ionic environment.

Salmonids show a spectrum of migratory types which range from freshwater species that are relatively sedentary to species which migrate between lakes and rivers, rivers and oceans, or rivers, lakes and oceans (Hoar, 1976). Generally, members of the genus *Oncorhynchus* (the Pacific salmon) have a

seaward migration followed by a period of growth in the sea which is then succeeded by an anadromous spawning migration. The seaward migration of juvenile salmon is a critical stage in the fish's life history: the salmon that migrate are generally small and have a correspondingly large surface area to volume which is disadvantageous when having to adjust to an environment that has changed from being hypoosmotic relative to plasma to one which is hyperosmotic.

Prior to, or accompanying the ocean migration, juvenile salmon undergo a period of development which prepares them for their transition into sea water. This process is known as smoltification. It consists of a coordinated modification of a complex array of regulatory mechanisms which causes morphological, behavoural and biochemical changes in the fresh water parr, transforming it into a smolt capable of withstanding sea water transition (Hoar, 1976; Folmar and Dickhoff, 1980; Gorbman *et al.*, 1982; Loretz, 1982). With the changes in salinity tolerance there are changes in the functioning of a number of osmoregulatory organs including the gills, skin, kidney, urinary bladder and gut (Loretz *et al.*, 1982). There have been numerous investigations studying the changes coinciding with smoltification and seawater adaptation, and over the last decade several comprehensive reviews have been published (Hoar, 1976; Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980; Barron, 1986; McCormick and Saunders, 1987; Hoar, 1988).

Most of the work on smoltification and seawater adaptation and the physiological changes associated with these events has, in North America, concentrated on the coho salmon, *Oncorhynchus kisutch*, and in Europe, on the Atlantic salmon, *Salmo salar*. The other species of Pacific salmon such as sockeye and quinnat have been investigated, but to a lesser extent. In contrast to the salmonid research in North America and Europe, there has been no comprehensive investigation to date on the seawater adaptability of New Zealand sockeye and quinnat salmon. Seawater tolerances of North American sockeye and quinnat salmon can not be assumed to be the same for New Zealand salmon, since salmon stocks in New Zealand have been isolated from North American populations for close on 90 years. Moreover, environmental conditions - for example, spawning sites and water temperatures - have been shown to greatly influence the seawater adaptability of salmon, and these differ between New Zealand and North America. It is already known that New Zealand quinnat can successfully adapt to sea water as they have formed an anadromous population; however, sockeye have been landlocked for the past 86 years and although they originally came from a sea-run population their ability to adapt

successfully to sea water has not been fully investigated.

The seawater adaptability of salmon has direct relevance to the aquaculture industry, and in particular ocean ranching and sea-cage rearing where there is an ocean phase. With ocean ranching, the salmon are reared in fresh water and released as juveniles which migrate to the sea. The juveniles remain in the ocean where they grow and return as adults 2-4 years later. With sea-cage rearing, the salmon are reared in fresh water to a certain size and then transferred to cages in sea water where they are fed and grown to a marketable size. Salmon released in an ocean ranching operation have the option to remain in the river system, mouth or estuary before moving into the ocean. However, the transition of salmon from fresh water to sea water in a sea cage-rearing operation is very rapid and the salmon are generally not exposed to intermediate salinities. The timing of this type of sea water transfer is critical for the salmon to successfully adapt. The majority of the sea water transfers of sockeye and quinnat performed in this thesis involved an abrupt transfer from fresh water to sea water.

Aims

The main purpose of this thesis is to describe the seawater adaptability of New Zealand quinnat and sockeye salmon. In order to do this, various aspects involved in the transfer of salmon from fresh water to sea water have been investigated. One aim was to establish what conditions (with reference to the physiological state of the salmon resident in fresh water and environmental parameters) were needed for a successful seawater transfer and hence the factors that were responsible for an unsuccessful transfer.

Since the movement of salmon from low to high salinity involves marked physiological changes it is obvious that this transition must be stressful to the salmon, especially if the salmon are placed directly from fresh water into sea water. A major aim of this research was to view the seawater transfer as a stressor and the physiological reactions of the transferred salmon as partly a stress response. To help elucidate this, the physiological changes of salmon that had failed to adapt and those that had successfully adapted to sea water were compared.

Structure of the Thesis.

The thesis has been divided into discrete chapters. Chapter 2, General Methods, describes the fish stocks and the procedures involved with fish handling, transportation and maintenance. The sampling methods and assays used are also described in this chapter. Chapters 3 to 8 describe experimental results and are written in such a manner that they might be subsequently submitted as discrete publications. Chapter 3 presents information on the stress responses of quinnat and sockeye salmon exposed to a variety of stressors. Many of these experiments were designed as controls for the subsequent chapters. Chapters 4 and 5 describe the seasonal changes in the seawater adaptability of sockeye and quinnat salmon. The changes associated with the direct transfer of salmon from fresh water to sea water are described in Chapter 4. Chapter 5 details seasonal physiological changes that occur in salmon that are resident in fresh water and relates them to sea water survival. In Chapters 6 and 7, the effect of seawater temperature and the effect of rate of salinity change on salmon survival is addressed. Chapter 8 details the effect of successful and unsuccessful seawater transfers on the gill morphology of sockeye salmon and discusses some of the changes in morphology with respect to a stress response. Finally, Chapter 9 gives an overview of the thesis and a summary of the findings.

CHAPTER 2

General Methods

Fish Populations

Sockeye (*Oncorhynchus nerka*) and quinnat (*O. tshawytscha*) salmon (0+ to yearlings) were obtained from two salmon farms: a Ministry of Agriculture and Fisheries (Fisheries Research Division) experimental research station situated on a tributary of the Rakaia river, the Glenariffe stream; and a small, private, pond-rearing farm at Prebbleton, 14 km south of Christchurch.

The captive quinnat salmon maintained at the Glenariffe Salmon Research Station were reared from ova obtained from quinnat that had returned to spawn in the Glenariffe stream. The sockeye salmon present at the Glenariffe Station originally came from a landlocked population that spawned in Larch Stream, Lake Ohau. The sockeye at Glenariffe had been resident in fresh water and bred through several generations. The quinnat and sockeye salmon maintained at the Prebbleton hatchery originated from the Glenariffe stocks.

Fish Collection and Maintenance

From September 1985 to June 1986, sockeye and quinnat were collected at selected times from the Glenariffe or Prebbleton salmon farms and transported to the Edward Percival Field Station (University of Canterbury), Kaikoura, for seawater adaptability experiments. Fish were transported in large, sealed, dark-coloured polythene bags placed inside 80 litre tanks. At the hatcheries, salmon were quickly dip-netted from raceways or circular ponds and transferred into the polythene bags which contained fresh water from the hatcheries main water supply. Fish were stocked at a density of 20-30g of fish per litre. Oxygen was bubbled into the water through an air-stone for several minutes until a large oxygen rich space had formed inside the bag. The bags were sealed with rubber bands, leaving a good sized "oxygen reservoir" above the water level. If salmon were transported during the summer, either a layer of crushed ice was placed along the bottom of the tanks, or chilli-bin coolers were positioned in between and around the plastic bags to prevent the water temperature from increasing markedly. If possible, fish were transported during late afternoon or at night.

The trip from Glenariffe to Kaikoura was broken into two stages: a two hour journey from Glenariffe to Christchurch and a three hour journey from Christchurch to Kaikoura which occurred the following day. Fish were held overnight in the Zoology Department (University of Canterbury) aquarium, in tanks supplied with artesian water flowing in at a rate of 8-10 litres per minute. Only a single trip was needed to transport salmon from the Prebbleton hatchery to Kaikoura.

On arrival at the Edward Percival Field Station, salmon were released from the plastic bags into 80 litre holding tanks and the fresh water replaced. Initial attempts to maintain fish in running fresh water by dechlorinating the Kaikoura town supply proved unsuccessful due to the extremely high (and variable) levels of chlorine in the water. Subsequently all fresh water was obtained from a spring located at the Kaikoura Nurseries. The fish were held in static water which was completely replaced every 5-6hrs (4-5 times per day). All tanks were continuously aerated.

Sea water was supplied to the tanks via a flow through system, where it was pumped directly from the sub-littoral zone into header tanks and then delivered to the experimental tanks at a rate of 2-4 litres per minute before running to waste. The salinity of the sea water remained relatively constant at 34‰. Fish were maintained under the natural seasonal conditions of photoperiod and water temperature. Commercial fish pellets were fed to the salmon every second day.

Seawater Transfers - Experimental Procedures

After transportation to Kaikoura, the salmon remained in fresh water for a minimum of four days before transfer to sea water. All seawater transfers were conducted by adding sea water to the fresh water already present in the holding tanks. This eliminated any handling stress which would have occurred if salmon were netted and moved from one tank or salinity into another (see Chapter 3). Two types of seawater transfer were made. Salmon were either abruptly transferred from fresh water to sea water (direct transfer, Chapters 4-7) or they were exposed to a gradual increase in salinity (progressive transfer) (see Chapter 6). During the direct transfer of salmon, sea water was supplied rapidly to the tanks at a rate of 10-12 litres per minute. Fresh water was totally exchanged for sea water within 20 minutes (Fig. 1a). The salinity of the water was monitored with a refractometer and once it reached 34‰ (100% sea water) the flow of sea water was reduced to 2-4 litres per min.

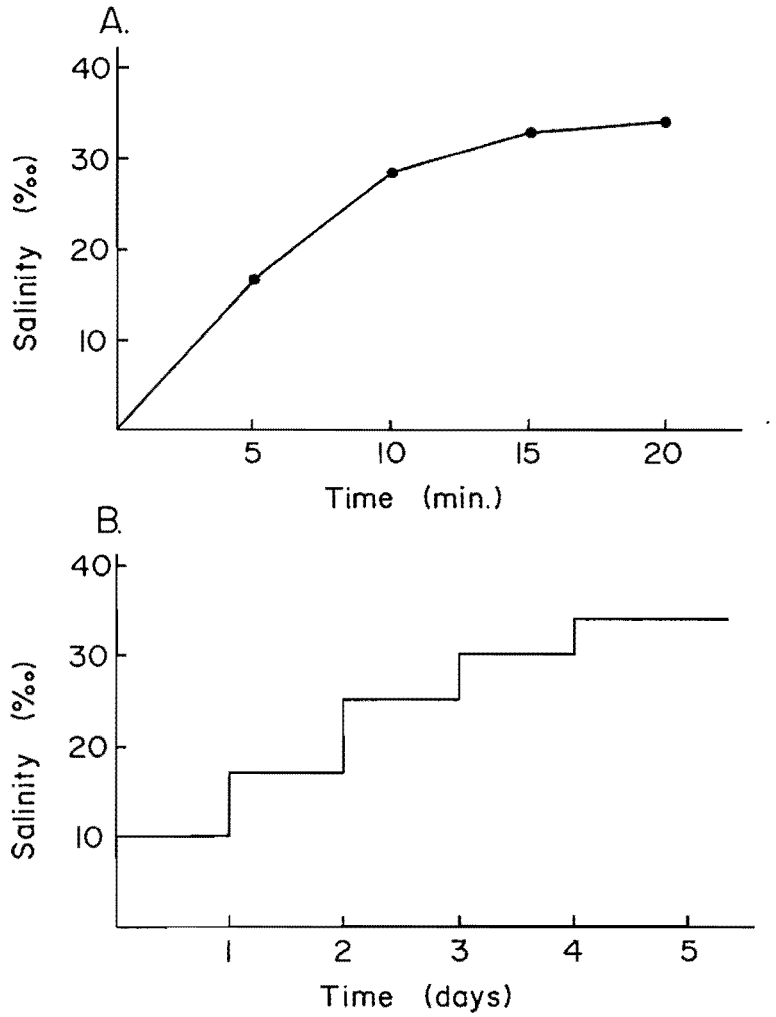

Figure 1A & B. Salinity changes in the tanks used for the direct transfer of salmon (A) and the progressive transfer of salmon (B).

The progressive transfer of salmon into 100% sea water was completed within five days. Due to the lack of flowing fresh water, the progressive transfer of salmon was conducted in well aerated static water. The salinity was increased in discrete steps (10‰,17‰,25‰, 30‰ and 34‰) at 24 hourly intervals. The desired salinity was achieved by slowly pouring buckets of sea water into the tanks which were monitored with a refractometer. Figure 1b illustrates the changes in salinity during a progressive transfer.

All seawater transfers were started between 9 00 and 10 00 hours. Salmon maintained in fresh water were used as controls during the seawater transfer experiments.

Sampling Procedures

At each sampling time, 6-10 fish were rapidly removed from a tank with a few sweeps of a dip-net and immediately placed into an anaesthetic solution (0.4% 2-phenoxyethanol). Care was taken during netting to cause the minimum disturbance to the fish remaining in the tank and no tank was sampled more than once in 24 hours. The solution of 2-phenoxyethanol was concentrated enough to completely anaesthetise the fish in less than 30 seconds. Fish were then serially removed from the anaesthetic, blotted on paper towels for 10-15 second to absorb any surplus water, weighed and their standard length (from the nose to the caudal peduncle) measured. Any changes in the external appearance and condition of the fish were noted.

Blood was taken from the caudal vasculature by severing the caudal peduncle immediately posterior to the adipose and anal fins. Before severance, excess mucus or fluid was wiped off the caudal peduncle with a tissue to minimise contamination of the blood sample. Blood samples taken from very small fish (less than 5g) were pooled and collected in a heparinised (ammonium heparinate) 1 ml plastic disposable syringe (27 gauge needle). These syringes had a negligible dead space which allowed small blood samples to be collected effectively. In larger fish, blood was collected in heparinised 1 or 2 ml plastic syringes (20 gauge needle). The larger diameter needle enabled blood to be collected more rapidly and reduced haemolysis of the red blood cells. To determine percentage haematocrit, samples of whole blood (10-30µl) were withdrawn into capillary tubes, sealed with plasticine and centrifuged at 20 000g. The tubes were measured immediately after centrifugation. Blood remaining in the syringes was transferred into polyethylene vials (Eppendorf 400µl centrifuge tubes) and centrifuged at 5000g for three minutes. The plasma was drawn off with a pasteur pipette and transferred into a new vial. All plasma samples were frozen and stored in a -80°C freezer until analysed. The total sample of fish (6-10) was bled within 5-7 minutes of net capture.

After the blood had been taken, gill arches were removed and either rinsed in homogenising buffer and frozen in vials for Na^+-K^+-ATPase activity determination, or were fixed in freshly prepared 2.5% glutaldehyde in 0.1M cacodylate buffer (pH 7.3) for morphological studies (see Chapter 7).

Finally, a sample of white myotomal muscle was taken from the dorso-lateral region of the salmon just behind the dorsal fin (Fig. 2). A sharp scalpel was used to separate the skin plus any red muscle from the white myotome.

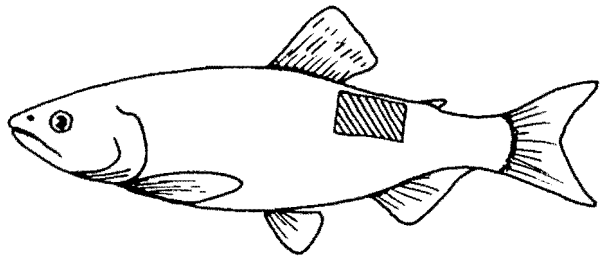

Figure 2. Lateral view of a salmon. Hatched area indicates the region where the white myotomal muscle was removed for water content determination.

Sample Analysis

The samples and measurements taken from the fish were analysed in a variety of ways. The methods for measuring the 'routine' parameters (e.g. plasma osmolarity, sodium and chloride concentrations, plasma cortisol, and gill Na^+-K^+-ATPase activity) are detailed below. The techniques that were specific to a particular chapter are described in that chapter's material and methods section.

Condition Factor

Length and weight measurements were used to calculate the condition factors of fish. Fish lengths and weights were too variable, and the sample size of 6-10 fish too small, for differences to be detected between samples as a result of the treatment, especially if the time interval was less than 96 hrs. The calculation of condition factors considerably reduced the variability between fish and enabled meaningful comparisons to be made between samples. Fulton's condition factor (K) was calculated for each fish:

$$K = \frac{100\ W}{L^3}$$, where

W = fish mass (g)

L = fish standard length (cm)

Plasma electrolytes and osmolarity

Plasma Na concentrations were analysed by atomic emission spectrophotometry using a Varian Techtron 1200 Atomic Absorption Spectrophotometer. Duplicate 5µl samples of plasma were diluted with 5mls of double distilled water and analysed immediately on the spectrophotometer. The concentrations in the

samples were estimated from calibration curves determined with NaCl standards. Plasma chloride concentrations were obtained from duplicate or triplicate 5-20µl plasma samples using a Radiometer CMT 10 Chloride titrator. Osmolarity was determined with a Wescor Inc. 5100C vapour pressure osmometer using 8µl quantities of plasma.

Muscle Water Content

Dissected white myotomal muscle samples were immediately placed onto a pre-weighed square of tin foil and weighed. The tin foil was wrapped around the muscle sample and placed in a 70° C oven until the dried weight was constant (approx. 48hrs). The percentage water content was calculated.

Cortisol Radioimmunoassay

Plasma cortisol was determined by radioimmunoassay (RIA) using tritiated cortisol ([1,2,6,7-^{3}H]cortisol, Amersham International) and cortisol antiserum obtained from Bioanalysis Ltd (U.K.). The antiserum was raised in sheep against a cortisol-3-(O-carboxymethyl)oxime-bovine serum conjugate and exhibited a high specificity. The percentage cross reactivity of various steroids, as supplied by Bioanalysis, is listed in Table 1.

Cortisol is generally considered to be the major corticosteroid in teleosts (Idler and Truscott, 1972), although recently, Weisbart and McGowan (1984) found in juvenile and adult Atlantic salmon that cortisone not cortisol was the major corticosteriod in this fish's plasma. Patino et al. (1987) also found that cortisone was similar to, or higher in concentration than cortisol in resting coho salmon, but concluded that cortisol was still the primary steriod secreted by the interrenal tissue and that cortisone arises primarily from peripheral conversion of cortisol to cortisone. No attempt was made in the RIA to separate cortisol (e.g. by thin layer chromatography) from other corticosteroids in the plasma. It was therefore essential that the corticosteroids present in appreciable concentrations (other than cortisol) in salmonid plasma had low cross reactivities. For example, both cortisone and 11-deoxycortisol can occur in reasonably high concentrations in the plasma of salmonids (Idler and Truscott, 1972; Sandor, 1979), however, they have low percentage cross reactivities to the antiserum (0.4%, 3.3% respectively) and so do not significantly affect the specificity of the assay. Reproductive steriods that can occur in reasonably high concentrations in fish and are not found on the cross-reactivity list provided by Bioanalysis (Table 1) were not considered important as all fish used in this study were immature. The cortisol RIA

TABLE 1. Percentage cross-reactivities* of various steroids to the cortisol antiserum (Bioanalysis).
* the ratio of cortisol concentration (by weight) to the concentration of the cross reacting substance which results in a 50% displacement of radioactive cortisol from the antiserum
i.e. [(pg cortisol)/(pg of steroid)]X100

Cortisol	100.0%
21-Deoxycortisol	65.0%
Fludrocortisone	9.6%
Prednisolone	6.9%
17 -Hydroxyprogesterone	5.0%
11-Deoxycortisol	3.3%
Cortisone	0.4%
Corticosterone	0.2%
11-Deoxycorticosterone	<0.2%
Progesterone	<0.2%
Dexamethasone	<0.2%

protocol used was an adaptation of the methods suggested by Bioanalysis. Each step of the assay was checked and if necessary altered to achieve better sensitivity and precision.

Chemicals

All components of the assay were reconstituted in a 0.01M phosphate buffered, 0.9% saline that contained 0.1% (w/v) gelatin, pH 7.4. The antiserum arrived freeze dried. On receipt, the antiserum was reconstituted in 1ml of distilled water and then further diluted to 10ml with assay buffer to provide a stock solution that could be easily divided into convenient aliquots and frozen at -80°C until needed. This cortisol antiserum stock solution was then diluted with buffer until the antiserum bound approximately 50% of labelled cortisol in the absence of unlabelled cortisol. The tritiated cortisol ([1,2,6,7-3H]cortisol, 250 mCi/mg) was diluted with buffer to give a working concentration of 0.25 µCi/ml. A stock solution of non-radioactive cortisol (Sigma Chemical Co. Lot No. 20F-0541) prepared in absolute ethanol was used for constructing standard curves.

Dextran coated charcoal was used to separate free cortisol from antibody-bound cortisol. Separation is dependent on the charcoal having differing affinities towards the free and bound cortisol. The charcoal preferentially absorbs the free cortisol but will, at greater concentrations, also absorb the antibody bound cortisol. Charcoal dose-response curves were developed for the assay to determine the amount of charcoal needed for efficient separation. Dextran

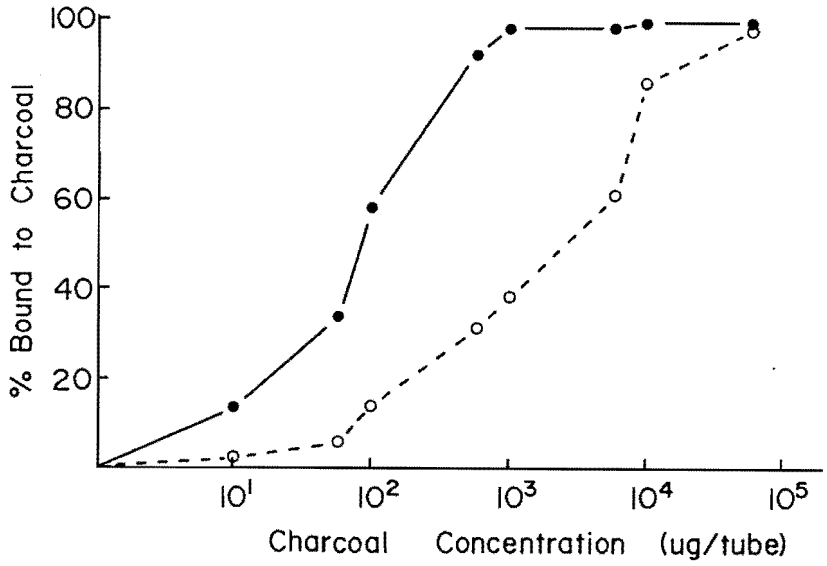

Figure 3. Dextran-coated charcoal dose response curves for the cortisol radioimmunoassay to determine the concentration of charcoal needed for efficient separation of the free from the bound cortisol. (●) free cortisol. (○) antibody bound cortisol.

coated charcoal was used since the dextran makes the charcoal "stickier" and permits easier centrifugation of the charcoal into a pellet (Binoux and Odell, 1973). Following the protocol suggested by Odell (1982), two series of tubes were set up. One series of tubes contained radiolabelled cortisol plus all other reagents used in the assay except the antiserum. The other series of tubes contained radiolabelled cortisol and excess antibody (a concentration at which >90% of the labelled cortisol is bound). Increasing amounts of dextran coated charcoal (10 - 50 000 µg charcoal) were added to the assay tubes. The tubes were mixed and incubated at 4°C for 1hr. They were then centrifuged at 1200g for 10 min, a sample of the supernatant taken for counting, and the percentage of labelled cortisol bound to the charcoal calculated. Two dextran charcoal dose response curves were plotted (Fig. 3) and a concentration of 1000µg charcoal/tube selected (for the RIA) for the efficient separation of the free from bound cortisol. At this concentration, the dextran charcoal suspension binds with approximately all of the free cortisol and only 38% of the bound cortisol. The charcoal suspension used for the RIA was prepared in assay buffer (1g activated charcoal and 0.1g Sephadex G25-150, swollen in buffer overnight was added to 500mls buffer). The suspension was continuously stirred at 4°C.

Radioimmunoassay

Frozen plasma samples were thawed and duplicate 10µl samples dispensed into plastic centrifuge tubes. A 50µl aliquot of absolute ethanol was added to the

tubes to precipitate the plasma proteins and to extract the cortisol. The tubes were vortex-mixed, left for 10min, and then centrifuged for 15min at 3000g (4°C). Between 20-40µl of supernatant was transferred into glass 12 X 75mm test tubes. Serial dilutions of the stock solution of non-radioactive cortisol (0, 10, 30, 50, 100, 200, 300, and 500 pg/10µl) were also dispensed in duplicate into glass tubes for determining a standard curve. The ethanol in all tubes was evaporated to dryness under a stream of dry nitrogen at 30°C.

One hundred microlitres each of antiserum and tritiated cortisol (approx. 40 000 DPM) were added to the tubes which were vortex-mixed and incubated at room temperature for 2hrs (or overnight at 4°C). Incubation times were checked to ensure that the assay had fully equilibrated within the predetermined time (Figs. 4a & 4b). The assay equilibrated after approximately 90 min and 3 hrs, at room temperature (approx. 20°C) and at 4°C, respectively. Following incubation, the protein bound cortisol was separated from the free cortisol by pipetting 500µl (1mg charcoal) of the chilled and stirred dextran charcoal into the tubes. The tubes were incubated at 4°C for 1hr and then centrifuged at 1200g for 10min to pelletise the charcoal. A 200µl sample of supernatant was taken for scintillation counting.

Liquid scintillation counting

The radioactivity of the samples was measured by liquid scintillation counting using a Beckman LS 2800 scintillation counter. Ten millilitres of a toluene-based scintillation cocktail (1000ml toluene; 500ml Triton X; 4g PPO, 0.4g POPOP) was dispensed into glass scintillation vials. The radioactivity of the vials was measured, and any vial that had an activity significantly greater than the background activity was discarded. The 200µl samples of supernatant were added to the precounted vials, mixed until the cocktail cleared, and then counted for 5 min. Total activity was determined by adding 100µl of tritiated cortisol and 400µl of assay buffer to the vials. All counts were converted to disintegrations per minute with respect to a tritium standard.

Calculation of plasma cortisol concentrations

Plasma cortisol concentrations were calculated by a computer program that is supplied with the Beckman scintillation counter. From the standards (0-500pg cortisol), bound cortisol (DPMs) was plotted against log dose (pg) and the standard curve fitted with a spline function. The programme then calculated the unknown plasma cortisol concentrations from the standard curve. As a backup and to check the accuracy of the programme, standard curves were

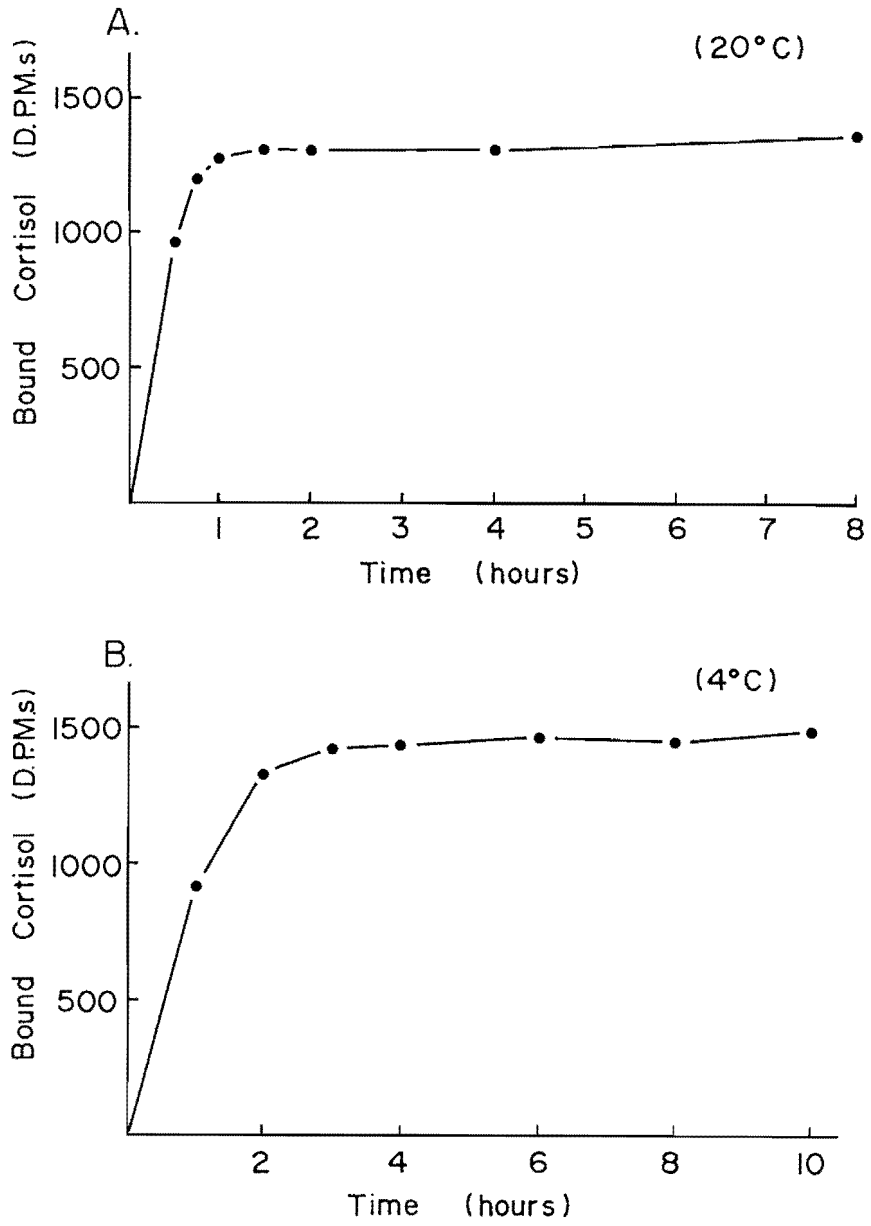

Figure 4A & B. Incubation curves for the cortisol radioimmunoassay. (A) Incubation at room temperature. (B) Incubation at 4 C.

plotted by hand, the cortisol concentrations of the plasma samples calculated, and these concentrations compared with the computer-generated results. There was no significant difference between the results derived from the two methods of calculation. A typical standard curve for the assay is shown in Fig. 5. Extraction of cortisol with ethanol resulted in a high recovery (>90%) with losses incorporated into the calculations.

Sensitivity

The minimum amount of cortisol detectable (distinguishable from zero) from the standard curve was 10pg cortisol, and for a 10ul plasma sample, an assay sensitivity of 2.5 ng/ml could be achieved.

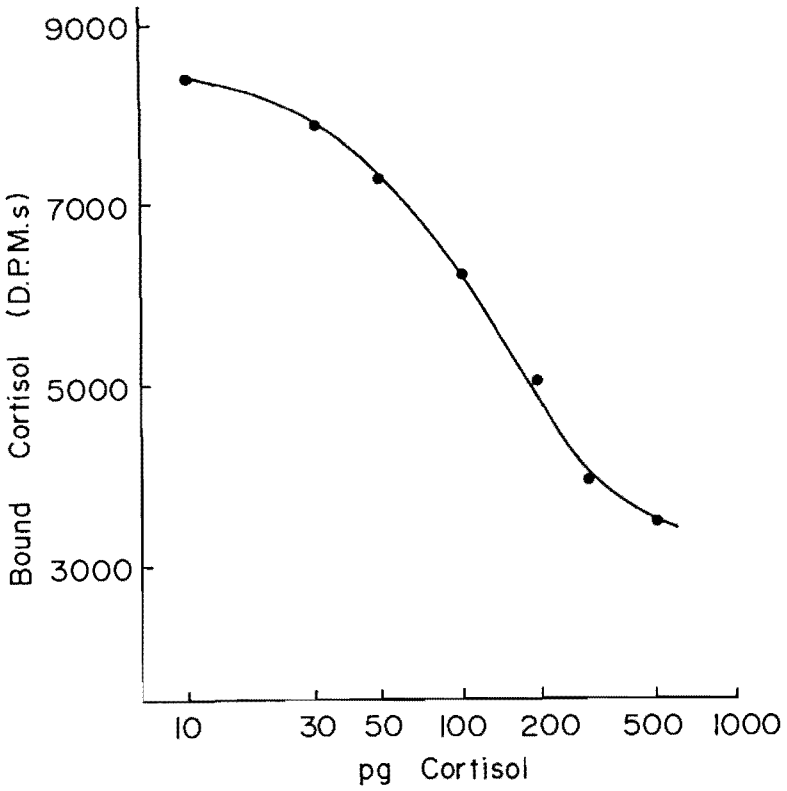

Figure 5. Standard curve for cortisol radioimmunoassay.

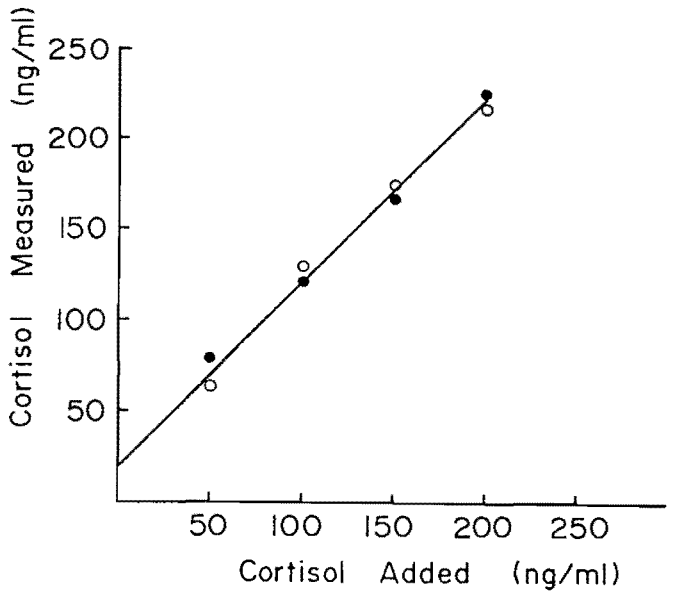

Figure 6. The radioimmunoassay recovery of cortisol added to a pooled plasma sample. Duplicate runs. (Linear regression, r=0.993).

Accuracy

To test the accuracy of the RIA, known amounts of cortisol (a concentration range of 0 to 250 ng/ml) were added to a plasma sample which was pooled from several salmon (but not stripped of endogenous cortisol). There was a high correlation (linear regression, r=0.993) between the amount of cortisol added and the amount of cortisol measured (Fig. 6).

Precision

A pooled plasma sample was used to determine interassay and intraassay variation. The mean intraassay (within an assay) and interassay (between assays) coefficients of variation for the RIA were 6.4% (n=4) and 10.2% (n=6), respectively. However, both the intraassay and interassay variation were influenced by concentration. Generally, the higher the concentration the smaller the variance.

Gill Na^+-K^+-ATPase Activity Assay

Methods to determine gill Na^+-K^+-ATPase activity have been described in detail by a number of workers (Zaugg and McLain 1970; Johnson et al. 1977; Zaugg 1982; and Langdon et al. 1984). In all of the procedures, the underlying principle of determining the specific activity of Na^+-K^+-ATPase remains the same. The enzymatic activity of Na^+-K^+-ATPase is quantified by measuring the amount of inorganic phosphate released when ATP is hydrolysed by the enzyme. Total ATPase activity (Na^+-K^+-Mg^{2+} -ATPase) is first quantified, then Mg^{2+}-ATPase activity alone was measured by selectively inhibiting the Na^+-K^+-ATPase enzyme with ouabain. The specific activity of Na^+-K^+-ATPase is calculated as the difference in the rate of inorganic phosphate liberated in the presence and absence of ouabain, per milligram of protein.

The various methods described in the literature differ in the degree of enzyme purification and in the procedures used to isolate the active microsomal fraction from the gill epithelium. Many of the methods require differential centrifugation and prolonged ultracentrifugation (Epstein et al. 1967; Thompson and Sargent 1977) to eliminate interfering substances and to purify the enzyme fraction. As noted by Zaugg (1982), the application of these methods on a large number of gill samples is time consuming and impractical. Without enzyme purification, there is a reduction in the specific activity of the Na^+-K^+-ATPase; however, this disadvantage is offset by the increase in the numbers of samples that can be processed.

The assay protocol used is an adaptation of the methods described by Johnston et al. (1977) and Langdon et al. (1984), where only a crude gill homogenate is analysed.

Assay

Frozen gill samples were thawed and the gill epithelium removed by either scraping the epithelium off the filaments onto a cold glass plate, or if the gill arches were too small, by cutting the filaments off the arch. The tissue was placed into approximately 10 vol of ice cold buffer (see Appendix 1) and homogenised in a motorised teflon pestle and glass homogeniser held in crushed ice. The tissue was homogenised with 15-20 complete strokes of the pestle at 600 rpm. Cartilaginous material was pressed to the bottom of the homogeniser or allowed to settle for 20-30 seconds, and 200µl duplicate samples (50-100 µg protein) were taken from the upper half of the homogenate. This eliminated some of the larger sized interfering gill tissue (gill material that was not completely homogenised) and ensured a homogenous sample for analysis.

Enzyme activity was measured using two reaction mixtures (Appendix 1) which were identical except that one contained 0.58mM ouabain. The incubation medium without ouabain determined the total ATPase activity, whereas the medium with ouabain determined the Mg^{2+} dependent ATPase activity by inhibiting the Na^+-K^+-ATPase. Stock solutions of the incubation media were prepared with all the reagents except ATP which was added just prior to determining the ATPase activity.

The 200µl homogenised gill samples together with the incubation media (800µl) were dispensed into 1.5ml Eppendorf centrifuge tubes, vortex-mixed, and incubated at 35°C in a shaking water bath for 30 min. The reaction was terminated by adding 200µl ice cold, 10% trichloroacetic acid (TCA), vortex-mixing and immediate placement of the vials into the freezer for 5 minutes to chill. The tubes were centrifuged at 5000g for 5 minutes to pelletise the protein and a 700µl aliquot of the supernatant taken for determination of inorganic phosphate by the method of Peterson (1978). Protein concentrations for the enzyme preparations were determined by a modification of the method of Lowry et al. (1951), using bovine serum albumin as a standard.

The specific activities of Na^+-K^+-ATPase are expressed as µmol phosphate released/mg protein/hour. A computer programme was used to calculate the Na^+-K^+-ATPase activities.

CHAPTER 3

Evaluation of the Physiological Responses of Quinnat and Sockeye Salmon to Acute Stressors and Sampling Procedures.

Introduction

Biological stress in fish has been extensively discussed in the literature (Pickering, 1981). Despite this, the actual definition of stress varies greatly between authors. Stress has been described either as the 'cause' or the 'effect' of a general homeostatic disturbance. The terminology that will be used in this paper is similar to that suggested by Seyle (1956). A stimulus that is potentially harmful to the fish and initiates a homeostatic imbalance will be called the stressor which is, in turn, responsible for the subsequent stress response of the fish. Therefore, in general terms fish suffering from the consequences of a stressor are stressed or shows symptoms of stress. It should also be noted that stress may be neurogenic (see below).

Seyle (1950) suggested that in mammals, a generalised, non-specific, physiological response can be elicited by a wide variety of adverse stimuli, a phenomenon which he termed the General Adaptation Syndrome (G.A.S.). The general applicability of this model to mammals and its extension to the responses of fish to a variety of stressors is debatable. Seyle further argued that it is perhaps best to view the stress response as being made up of two components: a generalised stress response (encompassed by the G.A.S.); and a response that is specific to particular stressors. Essentially, it is the combination of both that provides an adaptive mechanism enabling the organism to regain homeostasis.

The initial stress response includes the activation of certain endocrine systems, through neural input. In vertebrates, the stress response is centred around two endocrine systems: the interrenal or adrenocortical tissue which produces corticosteriods (chiefly cortisol) and is controlled by the hypothalamus-pituitary-interrenal (HPI) axis; and chromaffin tissue which

produces catecholamines (adrenalin and noradrenalin) and is chiefly controlled by direct neural stimulation of the tissue. Mazeaud *et al.* (1977) described the activation of these endocrine systems in teleosts as the primary stress response. Increases in the concentration of these circulating hormones leads to various physiological and biochemical changes, termed the secondary stress responses. These changes can include ionic and osmotic dysfunction, changes in stored and circulating metabolites, and a disruption of the immune system. Wedemeyer and McLeay (1981) extended the original concept of Mazeaud *et al.* (1977) to include one further stage or progression, the tertiary stress response, which includes long term effects like changes in behaviour, stunting of growth, increased susceptibility to pathogens and infection, and (in severe cases) death. A tertiary response is most likely to be the result of a chronic exposure to a stressor which is continual and beyond the physiological compensatory mechanisms of the fish. The majority of work on stress in fish has concentrated on exposure to acute stressors (e.g. Pickering *et al.*, 1982, Barton *et al.*, 1985), and experiments are terminated before full compensation is achieved by the fish. The primary and secondary physiological changes produced by acute stress are the focus of this study rather than the long term changes in the performance of the fish (changes in growth rates, mortality).

Schreck (1981) incorporates a psychological aspect to the stress response and suggests that a stress response is only elicited when a fish is "aware" of the stressor. A reduced stress response will result if the ability of the fish to detect a potentially harmful stimulus is lacking. Moreover, a reduced response might be the result of a disturbed homeostasis, induced by a non-detectable stressor. Schreck adds that the criteria for inducing a stress response in fish is that a stressor has to produce a state of fright and/or inflict discomfort or pain. Schreck and Lorz (1978) found that chronic exposure of coho salmon (*Oncorhynchus kisutch*) to lethal amounts of cadmium caused no elevation of plasma cortisol titres. Schreck (1981) suggests that "awareness" of the stressor is required for a stress response to occur and that the stress response is the result of the fish being frightened, or in a state of discomfort or pain. If the fish's "awareness" is eliminated, or if the stressor is undetectable as in the case of cadmium, no physiological response will result.

Changes in a variety of haematological parameters such as plasma cortisol, glucose and haematocrit have been used to reliably characterise and quantify a stress response in fish (Wedemeyer and Yasutake, 1987; White and Fletcher, 1986;

Carmichael *et al.*, 1984; Schwalme and Mackay, 1985). Obviously obtaining blood from fish results in an additional, acute stress response caused by the sampling procedures (i.e. net capture, anaesthesia, and blood extraction), so it is essential that the time course of changes in the various investigated parameters induced by handling is known. In this way, sampling stressors can be distinguished from the actual stressors examined. A number of studies have examined the stress response of fish to anaesthetics, sampling and handling (Oikari and Soivio, 1975; Strange *et al.*, 1977; Strange and Schreck, 1978; Barton, *et al.*, 1979). However, as sampling techniques, maintenance, and the type, or stock of fish used can vary greatly, it is important to validate the methods used in any experiment to be confident that the sampling methods do not significantly affect the results. Knowledge of both the immediate and long term responses of a fish to a stressor may be needed. When controlling for blood sampling procedures, measured variables that show an immediate response to the stressor (have the fastest rise time) are the best indicators. Conversely, the best indicators of recovery from a stressor are variables that take the longest time to return to pre-stress levels.

The objectives of this study are to characterise the stress responses to handling of quinnat salmon (*Oncorhynchus tshawytscha*) in fresh water and sea water, and to validate experimental handling procedures (blood sampling and transportation) for both quinnat and sockeye salmon (*O. nerka*). Changes in plasma cortisol are used to indicate the primary stress response, and a variety of secondary stress effects are monitored with emphasis on ionic and osmotic changes.

Materials and Methods

Fish Stock

Sockeye (*Oncorhynchus nerka*) and quinnat (*O. tshawytscha*) salmon (O+ year) were obtained from the M.A.F. Glenariffe Research Hatchery and transported to either the Zoology Department, University of Canterbury, Christchurch, or to the Edward Percival Field Station, Kaikoura, where they were housed in large 80 litre, opaque aquaria at a density no greater than 6-10g fish per litre. The animals were maintained in a photoperiod of 12hr light : 12hr dark at the University of Canterbury, and in a natural photoperiod at the Field Station. The fish were not fed during the experimental trials.

Sampling Procedures

To sample, salmon were rapidly netted out of the aquaria and placed into the anaesthetic, 2-phenoxyethanol, which was diluted with 30% seawater to a concentration that immobilised the fish within 30 seconds (4-6 mls 2-phenoxyethanol per litre water). The fish were taken from the anaesthetic, and excess water removed by blotting with paper towels. The caudal fin was severed with a sharp scalpel and blood collected from the caudal vessels into a 1ml heparinised (ammonium heparinate) hypodermic syringe. The extracted blood was transferred into a 400µl Eppendorf centrifuge vial and a subsample immediately withdrawn into a micropipette for haematocrit determination by centrifugation at 20 000g for 3 minutes. The remainder of the sample was centrifuged for 3 minutes at 5000g. Plasma was then withdrawn and stored in vials at -80°C until analysed. The plasma samples were analysed for cortisol, osmolarity, sodium and chloride. Plasma cortisol was measured by radioimmunoassay using tritated cortisol and antiserum from Bioanalysis Ltd. and the other parameters by methods described previously (see Chapter 2).

Unless noted otherwise, these sampling methods were used for the experiments outlined below. No greater than 8 fish were sampled at any one time and sampling was completed within 10 minutes from the time of net capture.

Confinement and Physical Disturbance

Quinnat salmon (mean weight = 26.7±8.5g) were transferred from the 80 litre aquaria into small, plastic aquaria that had barely enough water to cover the dorsal fin of the salmon. The fish remained in these aquaria for 10 minutes during which time they were continually agitated by a plastic net that was moved back and forth across the aquarium. The salmon were then transferred back into the original aquaria. Blood samples were taken before, and 1, 2, 4, 12, 24, and 48 hours after the confinement and physical disturbance. This experiment was done using both freshwater- and seawater-adapted salmon. No mortality occurred during the stressor period and recovery.

Severe Heat Stress

The effect of a sudden heat shock as a severe stressor to sockeye salmon was investigated. Freshwater sockeye (mean weight = 18.3±4.4g) were netted and transferred from an aquarium set at 16°C to one set at 35°C where they remained for ten minutes before being placed back into the original temperature to recover. Samples were taken before, and 1, 3, 12, 24, and 48 hrs after the heat treatment. Some mortality occurred during the 48hr recovery period. Of

the 37 fish stressed, five died within the first hour, and three died between 24-48hrs after treatment.

Short term effects of blood sampling

The following experiments were designed to evaluate the short term effects of blood sampling on the stress response of quinnat and sockeye salmon. For both species seven salmon were netted and then anaesthetised. The salmon were removed from the anaesthetic, placed onto a moist paper towel, and then sampled at 1, 3, 5, 8, 12, 15, and 25min after the initial net capture. This procedure was replicated three times for each species.

To evaluate the effect of the repeated sampling of fish held in the same aquarium, sub-groups of salmon were sampled at 0, 1, 2, 12, and 24hrs. Thus fish in later sub-groups were disturbed as earlier sub-groups were sampled.

Transportation

The effect of transportation was examined in both sockeye and quinnat salmon. Fish were transported from the Zoology Department, University of Canterbury, to the Edward Percival Field Station, Kaikoura, in March 1985. Fish were netted from the Zoology Department aquaria and placed into large plastic bags filled with oxygen saturated water. The fish were stocked at a density of 20-30g fish per litre. The bags were sealed with an oxygen filled space and transported to Kaikoura. The water temperature in the plastic bags was initially 16° C and increased to 17.5 °C during the three hour trip to the field station. The fish were unloaded into 80l tanks at a density of 6-10g fish per litre to recover. Blood samples were taken before, and immediately after transportation, and after 12, 18, 36, and 84hrs of recovery.

Statistical Analysis

Results are presented as the mean±S.E. Where appropriate, results were analysed with the "Teddybear" statistical computer package, which incorporates Analysis of Variance (ANOVA) and the Duncans Multiple Range Test. Some data was normalised with a logarithmic transformation before the statistical analysis was valid.

Results

Confinement and Physical Disturbance

Both freshwater- and seawater-acclimated quinnat salmon showed a rapid and significant increase in plasma cortisol when confined and physically disturbed with a net ($p < 0.01$, Fig. 1A and 1B). The cortisol response in both cases was recorded as peaking at 1hr followed by a rapid return to control levels within 4hours. However, changes in osmolarity, haematocrit, and sodium and chloride concentrations were different in the freshwater- and seawater-acclimated salmon. In fresh water, the confinement and agitation induced a significant decrease in plasma sodium and chloride concentrations, and blood osmolarity (Fig. 1A), whereas in seawater there was a significant increase in sodium and chloride levels and osmolarity. Both the absolute and percentage changes in sodium and chloride concentrations were greater in seawater-adapted salmon. For both the seawater- and freshwater-adapted salmon, the recovery time for the electrolytes was longer than for cortisol, with the electrolytes returning to baseline values between 12 and 24hrs. Haematocrit increased significantly only in the seawater-adapted salmon, increasing from 37.4±0.6% to 42.2± 1.3% (4.8 unit increase).

Severe Heat Stress

Freshwater-acclimated sockeye exposed to a sudden heat shock displayed an increase in general activity and ventilation rate and, after 10min at 35°C, several of the fish appeared exhausted and lay on the bottom of the aquarium with a ventilation rhythm that was slow but deep. Physiologically, the salmon exhibited a marked increase in both haematocrit and plasma cortisol (Fig.2). Plasma cortisol increased about 15-fold and peaked after three hours. Haematocrit peaked after 1hr and it took 24hrs for both the haematocrit and cortisol to return to baseline values.

Short term effect of blood sampling

There was no significant difference in the plasma cortisol titres of quinnat salmon sampled within 12 minutes of netting and anaesthesia (Fig. 3). There was no change in plasma cortisol concentrations of sockeye salmon during the 25 minute sampling period (Fig. 3). Twenty five minutes after being netted and anaesthetised, the plasma cortisol concentration of quinnat salmon had increased to 48±10 ng/ml cortisol, about a three fold increase from the initial plasma cortisol levels. The elevation of plasma cortisol levels was faster and larger for quinnat salmon than for sockeye.

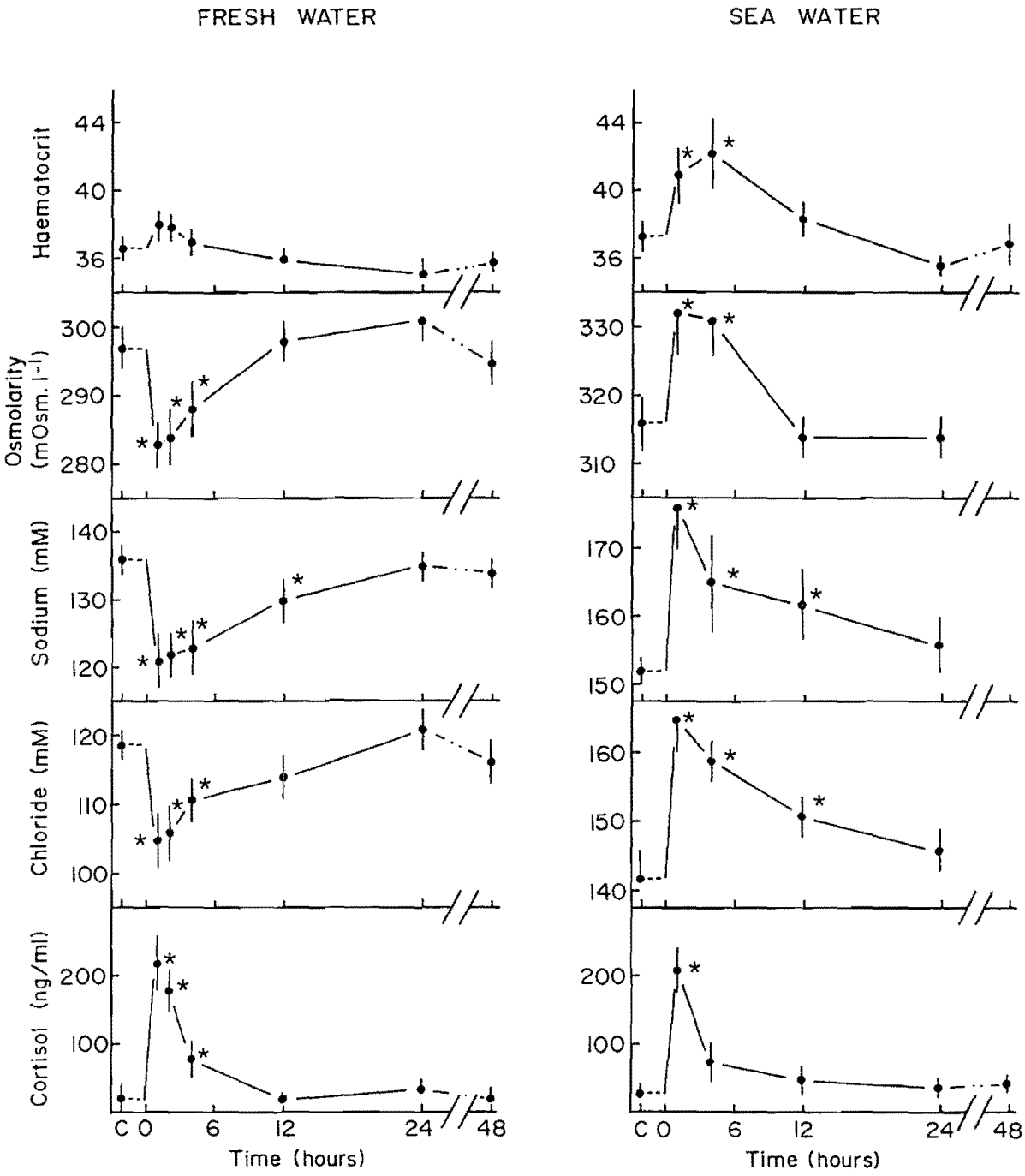

Figure 1. Changes in haematocrit and plasma variables subjected to intense handling and confinement for 10 minutes. A. Freshwater acclimated quinnat salmon. B. Seawater acclimated quinnat salmon. Values are means±S.E. (n=4-6). Asterisks indicate mean values that are significantly different from initial values (Duncan's multiple-range test, $P<0.05$).

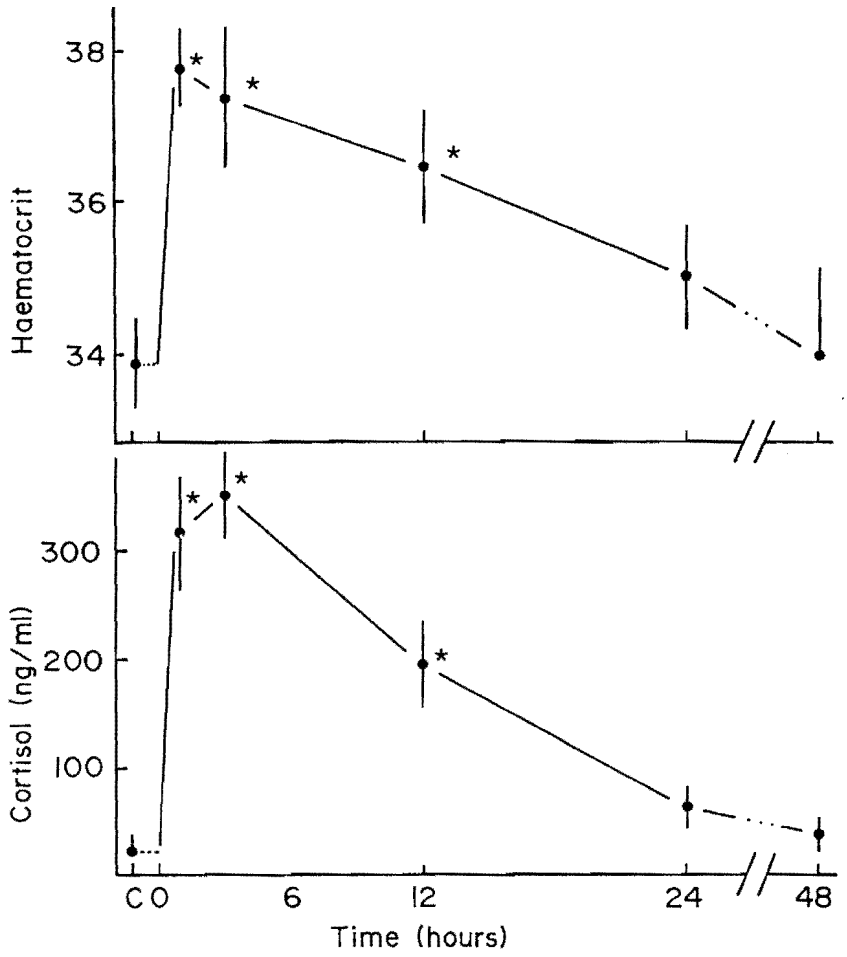

Figure 2. Changes in plasma cortisol concentrations and haematocrit of freshwater acclimated sockeye salmon subjected to a 10 minute temperature stressor (increase from 16 C to 35 C). Values are means±S.E. (n=3-6). C - control sample prior to temperature stressor. Asterisks indicate mean values that are significantly different from initial values (Duncan's multiple-range test, $P<0.05$).

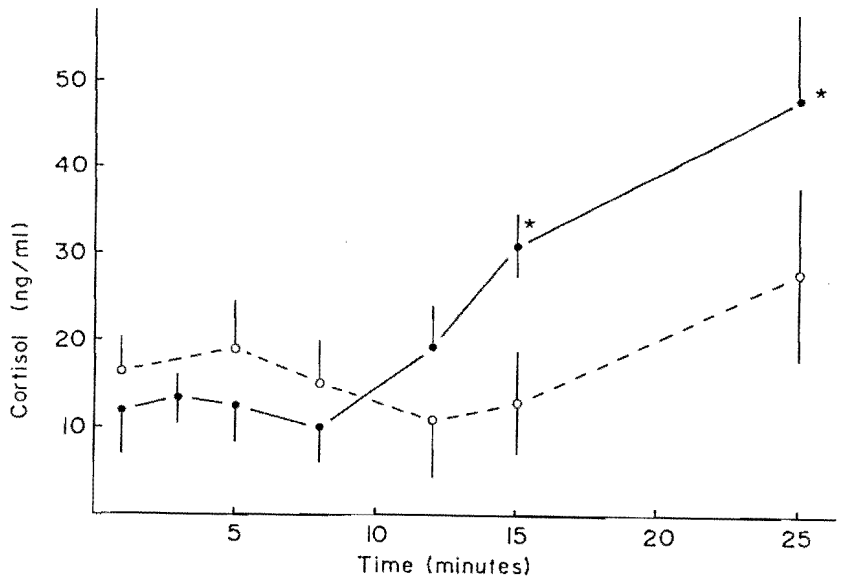

Figure 3. Changes in plasma cortisol levels of sockeye (○) and quinnat salmon (●), that have been netted and anaesthetised with 2-phenoxyethanol. Values are means±S.E. (n=3). Asterisk indicates mean values are significantly different from initial value (Duncan's multiple range test, $P<0.05$).

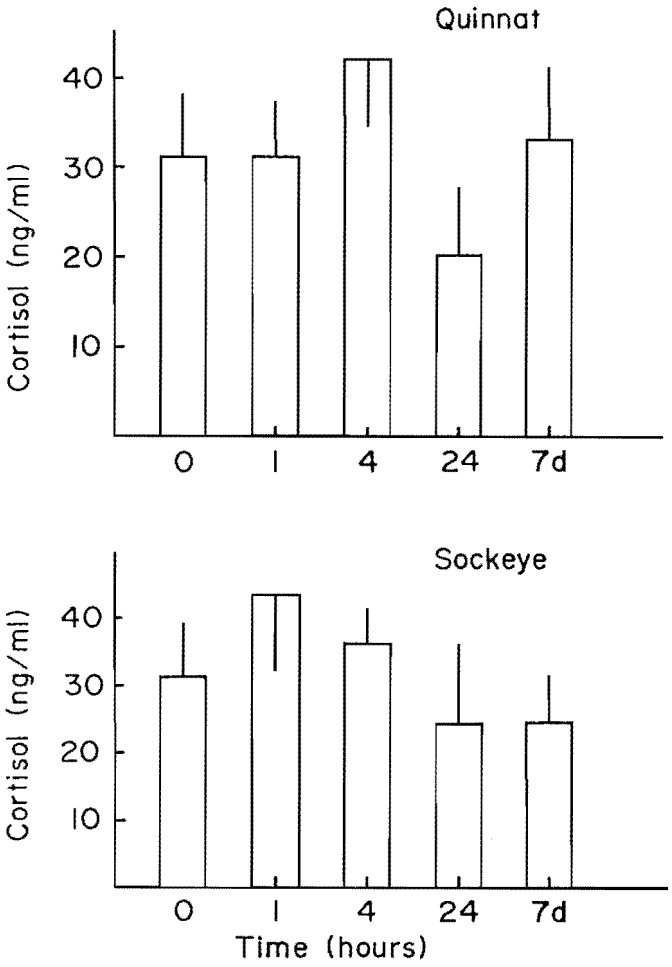

Figure 4. The effect of repeated sampling from an aquarium on the plasma cortisol levels of quinnat and sockeye salmon. Values are means±S.E. (n=5-7).

For both quinnat and sockeye salmon, there was no significant difference in the plasma cortisol concentrations of the fish sampled successively from the same aquarium at 0, 1, 4, 12, and 24hrs (Fig. 4A & B).

Transportation

On arrival at Kaikoura, both the quinnat and sockeye salmon did not appear unduly stressed after three hours of transportation. A few fish were taking the occasional gulp of air at the surface, and some scale loss had occurred, although this was negligible. The effect of transportation increased plasma cortisol concentrations by 100ng/ml in sockeye and 150ng/ml in quinnat salmon. Plasma cortisol concentrations returned within 18hrs to levels similar to those found in salmon prior to transportation (Fig. 5). A greater elevation of plasma cortisol occurred in the quinnat salmon, and the quinnat generally appeared to

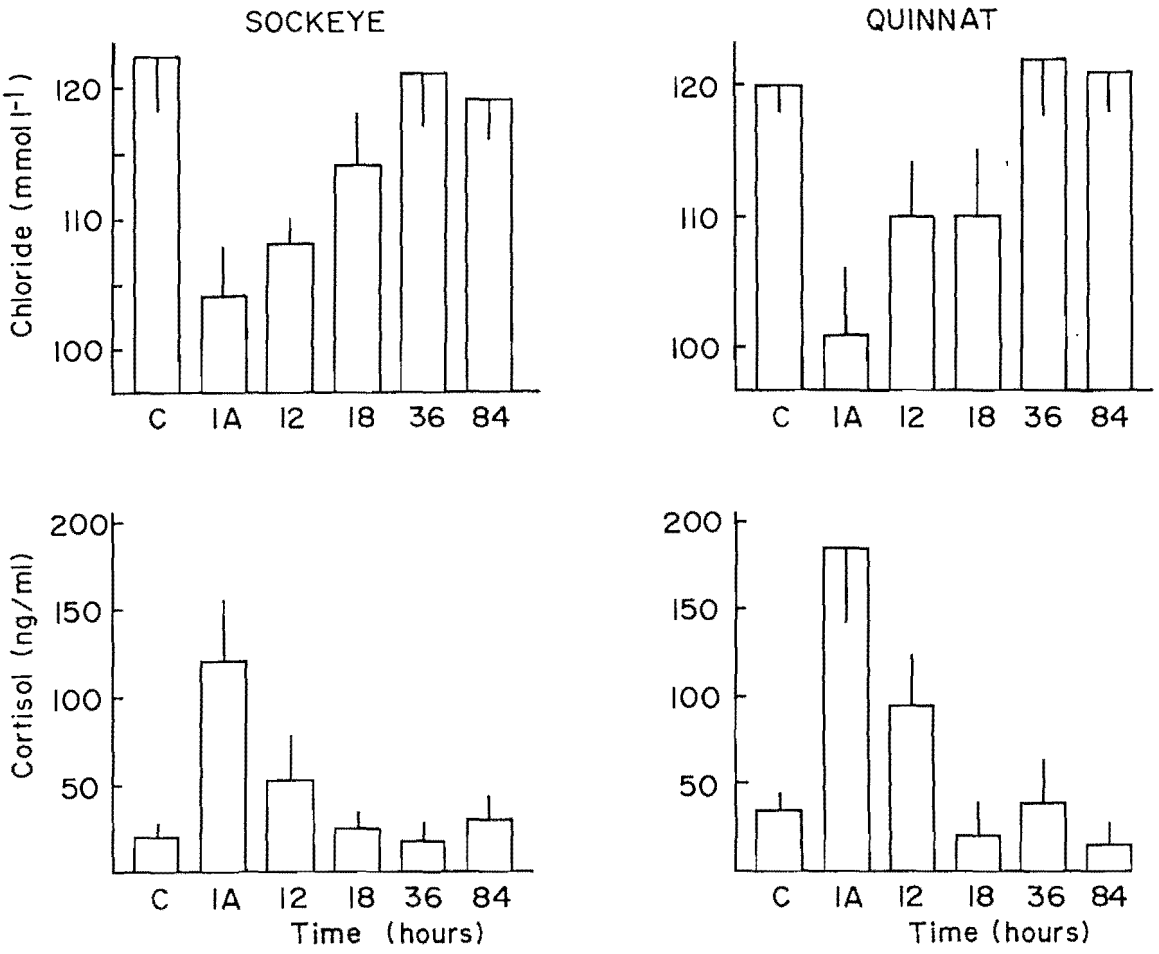

Figure 5. Changes in plasma cortisol and chloride concentrations in response to transportation. C = Before transportation. I.A. = immediately after transportation followed by hours of recovery. Values are means±S.E. (n=4-7).

be more distressed by transportation and handling. As in the freshwater confinement and agitation experiment, transportation of fish in fresh water caused a transient decrease in plasma chloride concentrations (Fig. 5). The recovery of plasma chloride took longer than blood cortisol, returning to baseline values within 36hrs.

Discussion

A transient elevation of plasma cortisol resulted when sockeye and quinnat salmon were exposed to acute stressors. Regardless of the nature of the stressor, whether it was confinement, heat shock, transportation, or netting and anaesthesia, a similar primary stress response occurred. This generalised reaction of an elevation in plasma cortisol following the application of an

acute stressor adds support to Selye's (Selye, 1950) argument of a common physiological stress response, at least at the primary stress response level.

A rise in plasma cortisol has been reported to occur in a number of different teleost species exposed to a variety of different stressors (for review, see Pickering, 1981). For this reason, cortisol has been widely used as an indicator of stress in fish. Donaldson (1981) went a step further and suggested that the response of the hypothalamic-pituitary-interrenal axis (HPI), which can be monitored by changes in plasma cortisol concentrations, could be used to quantify and compare different stressors. Differences do occur in the magnitude and duration of the plasma cortisol elevation and this variation can be related in part to the type of stressor applied. The heat shock applied to sockeye salmon was the most severe stressor (the death of salmon, absent from other stressor experiments, supports this contention) and this caused the greatest increase in plasma cortisol as well as the longest recovery time. Generally, the more severe the stressor, the greater the cortisol elevation. For example, salmonids exposed to a range of concentrations of environmental pollutants or toxicants typically show a dose-dependant increase in the corticosteroid stress response (Donaldson and Dye, 1975; Donaldson, 1981). The magnitude of the physiological stress response is also affected by the number of stressors applied. Barton *et al.* (1986) showed that the physiological stress response of juvenile quinnat salmon is cumulative if the salmon are exposed to more than one stressor. The stress response of teleosts have also been shown to be influenced by the water quality (Barton *et al.*, 1985a), the health of the fish (Sumpter *et al.*, 1986; Robertson *et al.*, 1987) and the developmental stage (e.g. smoltification of salmonids, Barton *et al.*, 1985b; Specker and Schreck, 1982; Leatherland, 1985).

Minor differences were noted between the stress response of quinnat and sockeye salmon in this study. The effect of transportation on sockeye salmon elicited a smaller primary stress response than for quinnat and their general behaviours were markedly different. The quinnat were more easily frightened or visibly seen to be stressed than sockeye salmon. For example, if quinnat were netted, their movements were more pronounced and they appeared to be more distressed. This could account for the greater primary stress response.

It should be noted that an increase in plasma cortisol in fish might not always be indicative of a stress response. Plasma cortisol concentrations have been reported to fluctuate in a circadian fashion in teleosts. Pickering and

Pottinger (1983), found a diel variation of about 20 ng/ml cortisol in brown trout *Salmo trutta*. They found that the plasma cortisol concentration was elevated during the hours of darkness, a phenomenon that has also been reported for the rainbow trout, *Salmo gairdneri* (Bry, 1981). Fluctuations of this magnitude would not be large enough to be detected by the experimental protocol used in this study, and so the presence or absence of a plasma cortisol circadian rhythm in quinnat and sockeye can not be verified. Strange *et al.* (1977) found no diurnal variation in the plasma cortisol levels of quinnat salmon, although this could also result from an insensitivity of experimental protocol. The plasma cortisol fluctuations that have been found to occur in salmon during a 24 hour period have been small (20-40 ng/ml cortisol) and fluctuations of this size would not have a noticeable effect on the plasma cortisol elevation that results from an acute stressor of the severity used in this study.

It should also be noted that an alteration in cortisol concentrations may not occur when a stressor is applied. Fish exposed to non-lethal chronic stressors may initially show a corticosteriod elevation which is followed by a return to basal levels and this could be due to either compensation for the stressor leading to a new homeostatic set point, or through interrenal exhaustion Pickering and Stewart, 1984).

The haematocrit, plasma sodium and chloride concentrations and plasma osmotic pressure of quinnat salmon were altered by confinement and agitation. These secondary stress responses had a smaller percentage change from pre-stressed values and generally had longer recovery times than the plasma cortisol changes associated with a stressor. An increase or decrease in plasma sodium and chloride concentrations and osmotic pressure after quinnat salmon were confined and agitated with a net was dependent on the external salinity. In fresh water, salmon are hyperosmotic to the medium and hence ions were presumably lost down the concentration gradient and water gained by osmosis. Conversely, in sea water, salmon are hypoosmotic to the medium and so there was presumably an influx of ions and outflux of water. A greater relative change in sodium and chloride concentrations occurred in the seawater acclimated salmon and this could be due to permeability differences in the two media. The osmotic and ionic changes could be the direct response to the stressor and/or the result of the adaptive mechanisms (i.e. the primary stress responses) initiated by the fish to combat the stressor. Mazeaud and Mazeaud (1981) suggest that the osmotic dysfunction that occurs when fish are stressed

is due to the release of catecholamines which have a direct effect on the fish's branchial permeability to water and ions. Adrenaline, which is elevated during stress (Butler *et al.*, 1978), has been shown to increase the permeability of the gill epithelium of teleosts to water and electrolytes (Pic *et al.*, 1974, 1975). Adedire and Oduleye (1984) found stress induced water permeability changes in the tropical cichlid, *Oreochromis niloticus*. Cortisol may also affect the ionic and osmotic balance of fish, by behaving as a mineralocorticoid. It might be considered that the increase in plasma cortisol concentration after a stressor is caused by the osmotic dysfunction. However, Redding and Schreck (1983) found that an osmotic imbalance was not a necessity for a rise in plasma cortisol. They found that coho salmon placed into an isoosmotic medium and then stressed by crowding showed no osmotic imbalance. Moreover, the time course of changes recorded here suggests that cortisol peaks and returns to basal levels more rapidly than the ionic and osmotic concentrations.

Confinement and agitation caused an increase in the haematocrit of the seawater-and freshwater-acclimated salmon, although the increase in haematocrit was greater in the seawater acclimated salmon. The reason for the increase in haematocrit can not be determined without additional information. Soivio and Nikinmaa (1981) suggested that the elevation could be due to erythrocyte swelling and/or result from the stress related changes in the osmotic balance of the blood. The larger increase of haematocrit in the seawater acclimated quinnat could therefore be the result of both erythrocyte swelling and an efflux of water concentrating the blood. In the freshwater acclimated quinnat, the smaller increase in haematocrit could have resulted from a positive increase such as the swelling of the erythrocytes being offset by a hemodilution of the blood through an osmotic influx of water. Beggs *et al.* (1980) found that in the freshwater muskellunge (*Esox masquinongy*), haematocrit levels were initially elevated and then were depressed after capture by angling. The reduced levels were the result of dilution of the blood.

Blood sampling-related stress responses were absent if the blood samples were taken within 10-12 minutes of netting and anaesthesia. Of the variables studied, plasma cortisol showed the fastest rise time and hence is the variable that needs to be monitored and controlled for during blood sampling. The influence of several types of anaesthetics and blood sampling methods on blood parameters has been reported (Oikari and Soivio, 1975; Strange and Schreck, 1978; Wells *et al.*, 1984). Strange and Schreck (1978) found that quinnat salmon anaesthetised rapidly with 100mg/l MS-222 (ethyl m aminobenzoate

methanesulfonate) had no effect on plasma cortisol concentrations. The fish were exposed to MS-222 in their aquaria and were not netted out and placed into the anaesthetic as was done in this study. The brief netting and placement into the anaesthetic could have caused the increase in plasma cortisol concentration, but it equally could be to a specific reaction of the fish to 2-phenoxyethanol.

The transportation of quinnat and sockeye salmon affected plasma cortisol and chloride concentrations. Similar alterations in plasma cortisol and chloride levels as well as changes in haematocrit, plasma glucose, osmolarity and other electrolytes have been observed in fish after transportation (Carmichael *et al.*, 1983; Robertson *et al.*, 1987). The decrease in chloride concentration after transportation is part of a general osmotic imbalance of the blood, which includes changes in sodium concentrations and osmolarity. This osmotic dysfunction has been shown to have a significant effect on the subsequent survival of transported fish (Barton *et al.*, 1980; Specker and Schreck, 1980; Robertson *et al.*, 1987). The transportation of fish in water that is isoosmotic to the fish blood has been found to negate the disruption of the osmoregulatory status of the fish and significantly reduce mortality (Carmichael *et al.*, 1984). However in these experiments there was no mortality during the transportation of the sockeye and quinnat salmon and this might be attributed to a low stocking density and short transportation time.

In summary, the rapid changes in the plasma cortisol concentrations of fish exposed to an acute stressor can be used effectively to characterise the initial effects of a stressor; however, changes in plasma cortisol would not be suitable for evaluating recovery from a stressor. As an indicator for recovery from a stressor, changes in plasma chloride concentrations would be more suitable.

CHAPTER 4

Physiological Changes during the Seawater Transfer of Quinnat and Sockeye Salmon: Comparison between Successful and Unsuccessful Adaptation

Introduction

Dramatic physiological changes occur when teleosts move from fresh water to sea water, or vice versa. This transition into a different salinity normally takes place as a migrational phase in the life cycle of the fish, and fish that exhibit this type of migratory behaviour are collectively known as diadromous (Dadswell *et al.*, 1987). Many juvenile salmonids migrate downstream to the sea as part of their life history and associated prior to, or during this migration, is a complex series of biochemical, physiological, morphological and behavioural changes which help to prepare the fish for its new osmotic environment. While still in fresh water the juvenile salmon partially pre-adapt to the marine habitat. This transformation or metamorphosis is known as the parr-smolt transformation or smoltification and has been extensively researched over the last ten years (see reviews by Folmar and Dickhoff, 1980; Wedemeyer *et al.*, 1980; Barron, 1986; Hoar 1988).

Intimately associated with smoltification is the increased ability of the salmon to survive in, and adapt successfully to sea water. The adaptation of salmonids to sea water has also been widely studied, and the majority of studies have concentrated on the physiological changes that occur when salmon successfully adapt to sea water (Boeuf *et al.*, 1978; Folmar and Dickhoff, 1979; Langdon and Thorpe, 1984; Redding *et al.*, 1984; Nichols and Weisbart, 1985; Fouchereau-Peron *et al.*, 1986; Sweeting and McKeown, 1987). For the transition from fresh water to sea water to be successful, the fish has to completely reverse its osmoregulatory mechanisms. In fresh water, the salmonid is hyperosmotic to its environment and so there is a continual loss of ions and influx of water which is countered by the fish actively absorbing ions and producing copious amounts of dilute urine. Conversely, a fish living in sea water is hypoosmotic to its surroundings and so to maintain homeostasis it must counteract the loss of water and influx of ions by actively transporting ions out and by obtaining osmotically free water (from ingested sea water where

the excess ions being removed via the gut, kidney/bladder and the gills.

Many factors can affect the survival of salmonids transferred to sea water. The salinity of the sea water, water temperature, age and size of the fish and developmental stage (pre-smolted, smolted, or post-smolted) have all been reported to have an effect on survival (Wedemeyer *et al.*, 1980). Differences may also exist between different species of salmon and between different stocks and brood years of the same species (Clarke, 1982). The mortality resulting from the seawater transfer of salmon can be more pronounced if the salmon are transferred directly from fresh water to sea water without passing through any intermediate salinities. This type of abrupt seawater transfer is practiced in most sea-cage rearing operations. The transition from fresh water to sea water is extremely rapid so if the environmental conditions are not right or the juvenile salmon not fully smolted, then high mortality may result.

The aims of this research are to characterise the physiological changes that occur when sockeye (*Oncorhynchus nerka*) and quinnat salmon (*O. tshawytscha*) are transferred directly from fresh water to sea water. Comparisons are made between fish that successfully and unsuccessfully adapt to sea water. Eight sea water transfers were made and physiological data collected for quinnat and sockeye salmon. However, to simplify analysis and discussion only three representative transfers for each species are given.

Materials and Methods

Fish Stock and Seawater Transfers

Sockeye and quinnat salmon were obtained from a Ministry of Agriculture and Fisheries salmon hatchery which is situated on the Glenariffe stream, a tributary of the Rakaia river. The fish were transported to the Edward Percival Field Station, Kaikoura, New Zealand where the seawater transfer experiments were performed. The salmon were housed under a natural photoperiod and in 80 litre tanks at a density no greater than 6-10g fish per litre. The transfer from fresh water to sea water was achieved within 20 minutes by exchanging the fresh water with sea water flowing at a rate of 10-12 litres per minute. All transfers were started between 0900 and 10 00 hours. Once a salinity of 34‰ was achieved, the flow of sea water was reduced to 2-4 litres per minute. Both quinnat and sockeye salmon were transferred into sea water in January, March and July 1986 as summarised in Table 1. Some salmon

Table 1. Water temperatures and mean weights of fish transferred directly from fresh water into sea water.

	Transfer Date	Fish Weight (g) (x ± S.D.)	F.W. Temp. (°C)	S.W. Temp. (°C)
Sockeye Quinnat	January 1986	23.1±3.4 9.2±1.9	15.2	17.6
Sockeye Quinnat	March 1986	58.0±13.2 30.2±5.6	14.2	16.8
Sockeye Quinnat	July 1986	95.0±13.0 70.9±11.2	11.4	10.5

were left in fresh water and acted as the controls. The aquaria were regularly checked for mortalities and the fish that had died were removed and weighed.

Sample Collection

Samples of fish were taken immediately before seawater transfer of freshwater adapted salmon, and 1, 2, 4, 12, 24, 48, 96 hours and 12 and 30 days after transfer. The freshwater controls were sampled at 0, 1, 4 and 24 hours, and after 7 days. Less control sampling times were used because of logistics and also preliminary studied over the previous year indicated no significant changes in freshwater controls. To sample, fish were rapidly caught with a hand net and placed into the anaesthetic, 2-phenoxyethanol, at a concentration (20mls per 5 litres of 30% sea water) that immobilised the fish within 30 seconds. The fish were removed from the anaesthetic, and the excess liquid removed with paper towels before the lengths and weights were noted, these to be used to determine Fulton's Condition Factor (C.F.= $100W/l^3$ where W=weight (g) and l=length (cm)). Immediately following this, the caudal fin was severed, and blood collected from the tail stump with a heparinised (ammonium heparinate) hypodermic syringe. A sample of blood was withdrawn into a glass micropipette, and centrifuged at 20 000g for 3 minutes to determine haematocrit (% red blood cells). The remaining blood was transferred into an Eppendorf tube and centrifuged at 5000g for 5 minutes. Plasma was withdrawn and stored at -80°C in sealed plastic vials until analysed. The blood samples (maximum of 7 fish per sample) were taken within 10 minutes of net capture. Gills were excised from the salmon, washed in a homogenising buffer and stored at -80°C until analysed for Na^+-K^+-ATPase activity (see Chapter 2 for details).

Blood and Gill Analytical Analysis

Plasma samples were analysed for sodium and chloride concentrations, osmolarity and cortisol levels. Chloride concentrations were determined from 20µl plasma samples with a CMT 10 Radiometer chloride titrator. Sodium concentrations were obtained from 5µl plasma samples diluted with 5mls water and analysed by emission spectrophotometry (Varian Techtron 1200 Atomic Absorption spectrophotometer). Osmolarities were measured with a vapour pressure osmometer (Wescor 5100C osmometer) using 8µl plasma samples. Plasma cortisol concentrations were determined by radioimmunoassay from 10µl plasma samples (see Chapter 2). Gill Na^+-K^+-ATPase activity was determined from a modification of the methods used by Johnston *et al.* (1977) and Langdon *et al.* (1984) (for details see Chapter 2).

Statistical Analysis

Results are expressed as mean ± standard error and were analysed for significance with the Student's t-test or with analysis of variance and a Duncan's multiple range test. Data sets that were not normally distributed were transformed.

Results

Mortality on Transfer

Sockeye salmon

Sockeye salmon transferred into sea water during January and March 1986 failed to adapt to the new environment and 100% mortality occurred within 5 days in January and within 4 days in March (Fig. 1a and b). A sigmoidally shaped survival curve resulted in both months. Few fish died within 24 hours of transfer, but then a sudden increase in mortality had occurred within the next 24 hours (48 hours post transfer). The smaller fish (by weight) tended to be the first to die, the larger fish surviving somewhat longer (Fig. 1a and b). No mortality occurred when sockeye were transferred into sea water in July 1986 (Fig. 1c). There was no mortality in the control sockeye that remained in fresh water during the sea water transfers.

Quinnat salmon

Quinnat salmon transferred from fresh water to sea water in January 1986 were unsuccessful in adapting, and no fish survived past 5 days (Fig. 2a). There was a rapid decrease in survival of the quinnat after seawater transfer. Twenty

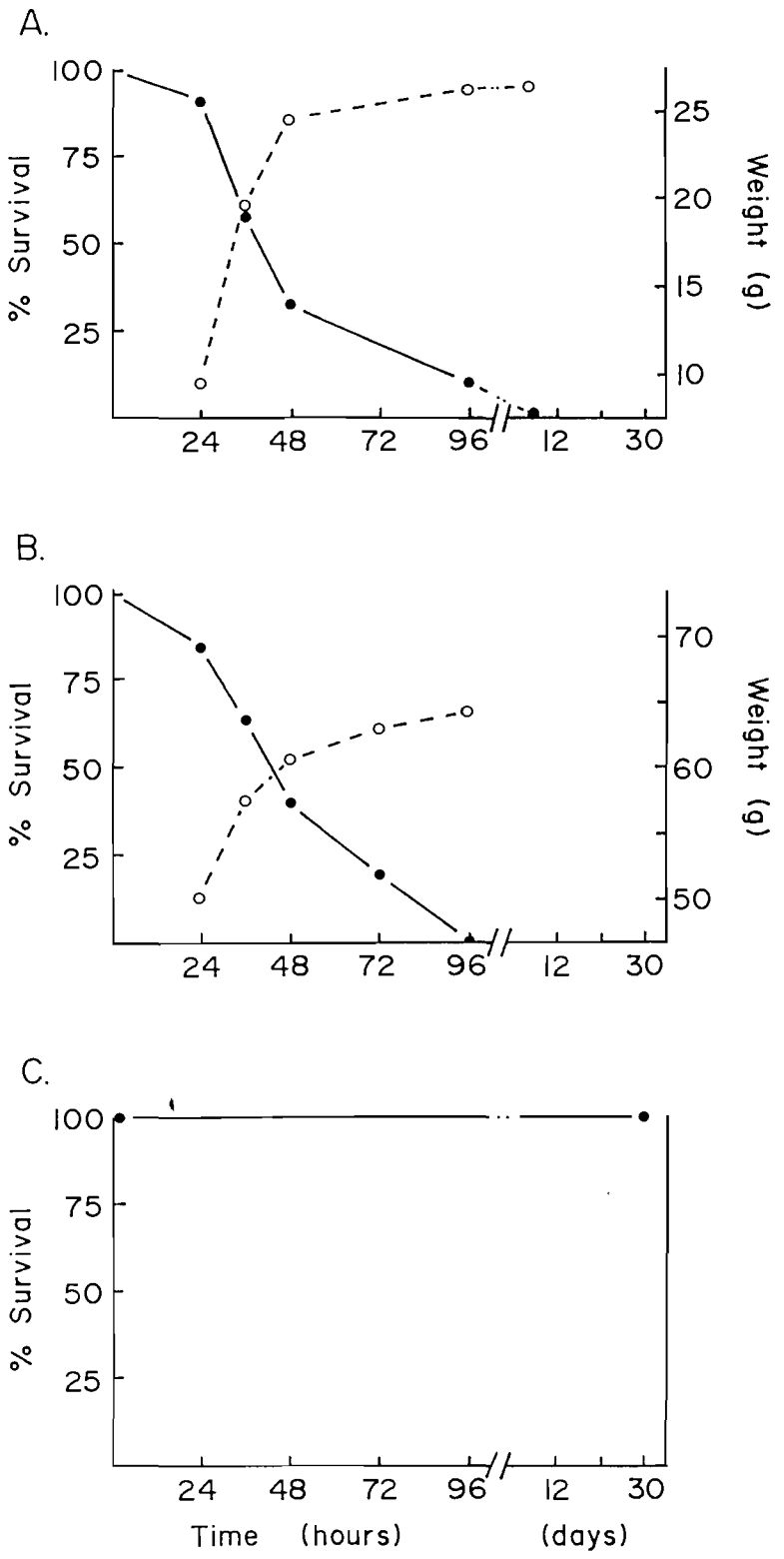

Figure 1. Survival curves and mean weight of dead sockeye salmon transferred directly into sea water in (A) January 1986, (B) March 1986 and (C) July 1986.

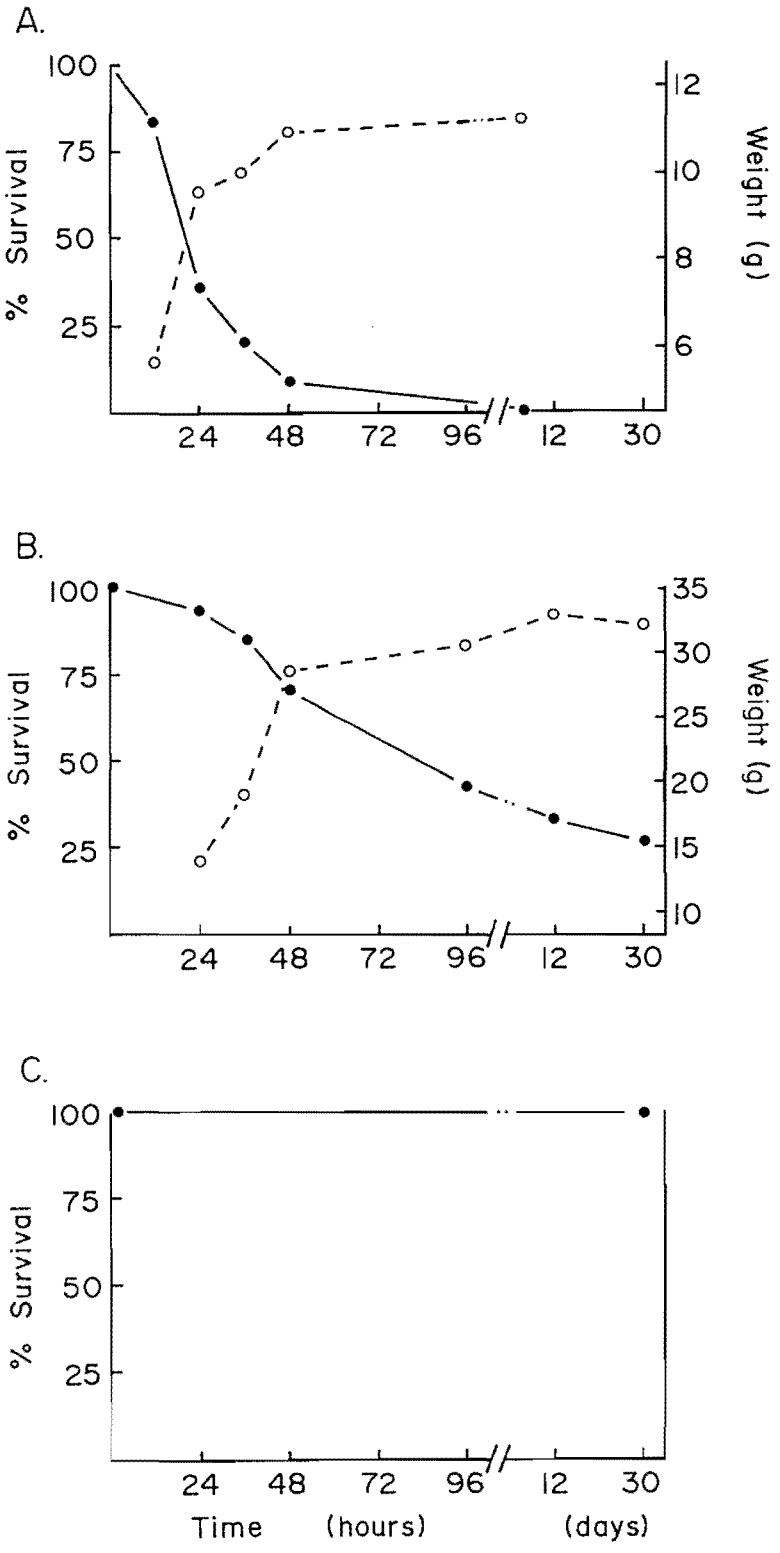

Figure 2. Survival curves and mean weight of dead quinnat salmon transferred directly into sea water in (A) January 1986, (B) March 1986 and (C) July 1986.

four hours post transfer, there was 60% mortality and by 48 hours only 6% of the quinnat transferred were alive. In March, only 26% of the fish survived the seawater transfer by 30 days (Fig. 2b). The majority of the mortalities occurred during the first 96 hours after transfer. Of the fish that had survived 30 days in sea water, some appeared moribund and stunted. The mortalities that occurred in both January and March were weight related. The smallest fish tended to die early, and the larger fish either took longer to die or were the fish that were alive after 30 days in sea water as in the March transfer . No mortality occurred in the quinnat transferred into sea water in July. There was also no mortality in the freshwater controls.

Physiological Changes During Seawater Transfer

Sockeye Salmon

Successful Seawater Adaptation

The transfer of sockeye to sea water resulted in an increase in plasma cortisol concentration that was both rapid and large (Fig. 3). Cortisol levels rose approximately 7-fold, from 33.5±8 ng ml^{-1} pre-transfer (basal) to a maximum recorded concentration of 231±23 ng ml^{-1} at 1 hour ($p<0.001$). This was followed by a somewhat slower return to pre-transfer levels, so that by 24 hours the plasma cortisol concentrations of the seawater-adapted salmon did not differ significantly from the freshwater sockeye values.

Plasma osmolarity, and sodium and chloride concentrations also increased after the seawater entry of sockeye (Fig. 3). Concentrations of sodium and chloride significantly increased from basal levels of 140±1 and 120±3 mmol l^{-1}, respectively, to a recorded maximum for sodium of 170±3 mmol l^{-1} and for chloride of 152±3 mmol l^{-1} (both $p<0.01$) at 24 hours. A peak in plasma osmolarity occurred at 48 hours post transfer. Thirty days after seawater transfer the plasma osmolarity, sodium and chloride concentrations were all significantly higher than those in freshwater-adapted sockeye, but had decreased significantly from the maximum values recorded at 24 hours ($p<0.05$). Haematocrit had increased 12 days post-seawater transfer (Fig. 3), rising from 35.0±0.8% to 40.5±1.3% ($p<0.01$) and remained elevated for the rest of the experimental trial. The condition factor of the fish did not change significantly after seawater transfer.

Gill Na^+-K^+-ATPase activity of sockeye was initially 4.03±0.99 µmol phosphate mg $protein^{-1}$ hr^{-1} in fresh water, but increased to 13.65±1.21 µmol phosphate mg $protein^{-1}$ hr^{-1} after 30 days ($p<0.01$) (Fig. 3).

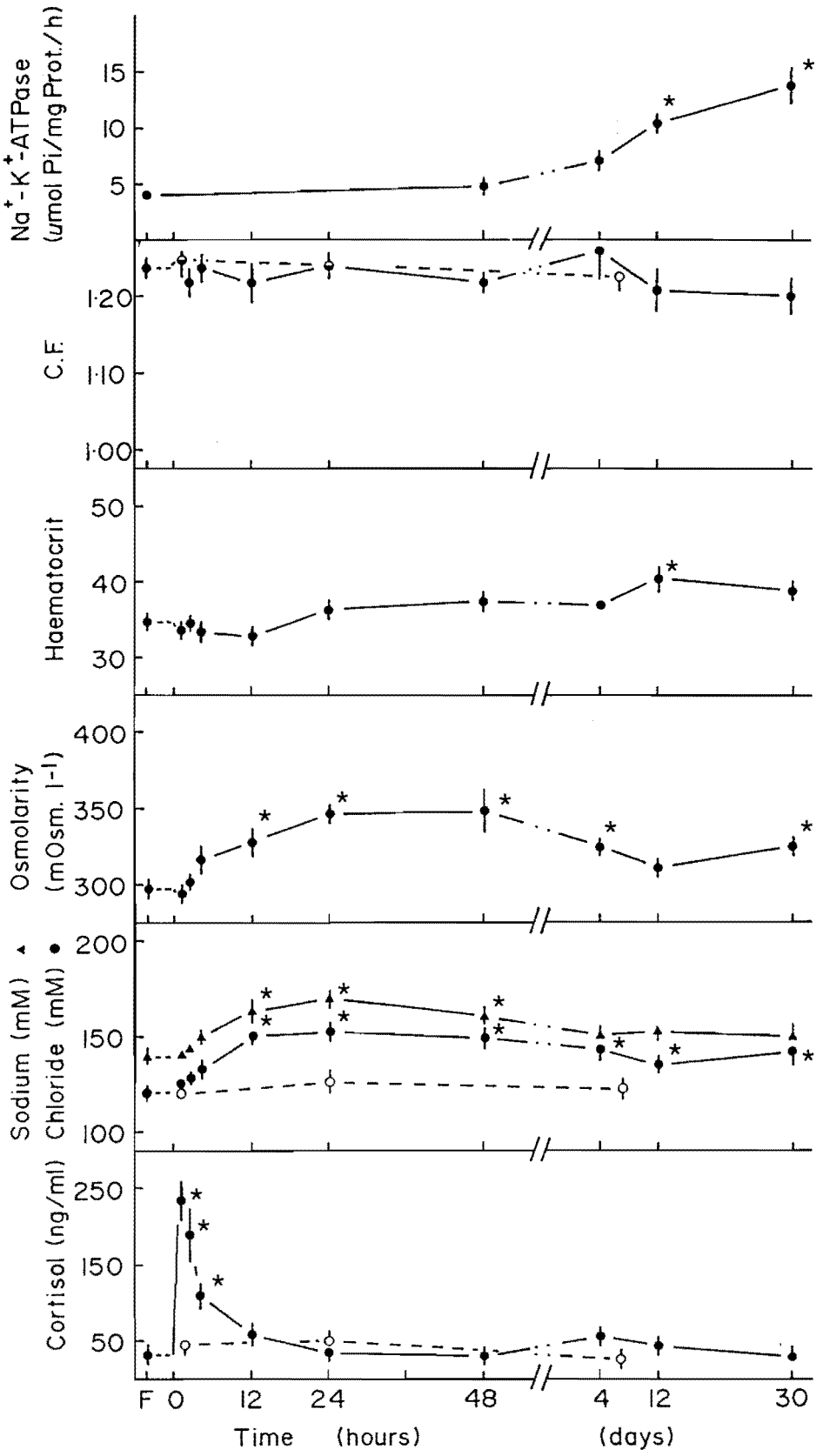

Figure 3. Physiological changes in sockeye salmon following a successful transfer to sea water in July 1986. F = initial freshwater sample. (O) freshwater controls. All values are means±S.E. Asterisks indicate a significant difference from initial freshwater sample ($P<0.05$).

Unsuccessful Seawater Adaptation

Sockeye transferred in January and March failed to adapt to sea water, similar physiological changes occurring in both transfers (Figs. 4 and 5). Plasma cortisol concentrations increased rapidly after transfer and were significantly greater than the freshwater levels for all of the seawater samples ($p<0.01$, Figs. 4 and 5). Cortisol concentrations did not return to basal levels as they did in the successful July salmon transfer, although the final concentrations were lower than during the earlier stages of transfers. The cortisol levels in the March seawater sampled salmon were generally higher than the January seawater sampled sockeye.

Plasma osmolarity and sodium and chloride concentrations increased after the seawater entry of the unsuccessful sockeye (Figs. 4 and 5). Compared with the July transferred salmon (Fig. 3), a larger and more rapid osmotic imbalance occurred in January and March. Osmotic pressures reached higher levels in March than January. Plasma osmolarity and chloride levels appeared to be still increasing after the final 72 hour sample in March, whereas in January there was no further increase after 24 hours post-transfer (Figs. 4 & 5).

In the January and March transfers there was an increase in haematocrit 12 hours post-transfer (Figs. 4 and 5). There was a more rapid, and generally a greater, deviation in haematocrit away from basal levels in these seawater transfers compared to the successful July transfer of sockeye. The condition factor of sockeye decreased markedly on seawater entry. In January and March the condition factor decreased by 18.0% and 22.6 % respectively, whereas in July the condition factor only decreased by 3.2%. There was no increase in gill Na^+-K^+-ATPase activity in the sockeye that failed to adapt to sea water in January 1986.

Freshwater Controls

There was no significant change in haematocrit and plasma cortisol and chloride concentrations in the freshwater control sockeye sampled in January and July (Figs. 3 and 4). The condition factor of these fish also remained unchanged.

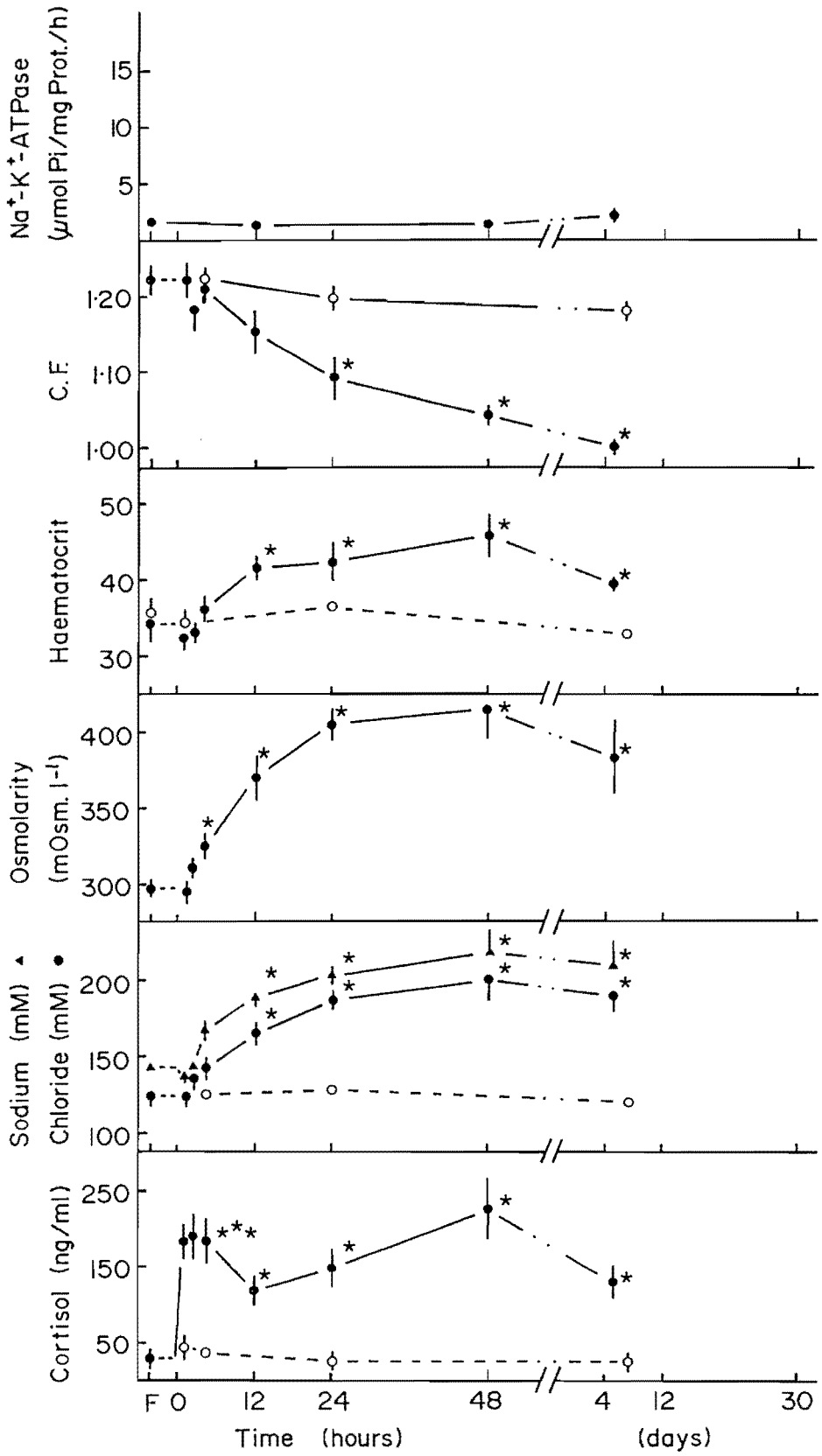

Figure 4. Physiological changes in sockeye salmon following an unsuccessful transfer to sea water in January 1986. F = initial freshwater sample. (O) freshwater controls. All values are means±S.E. Asterisks indicate a significant difference from initial freshwater sample ($P<0.05$).

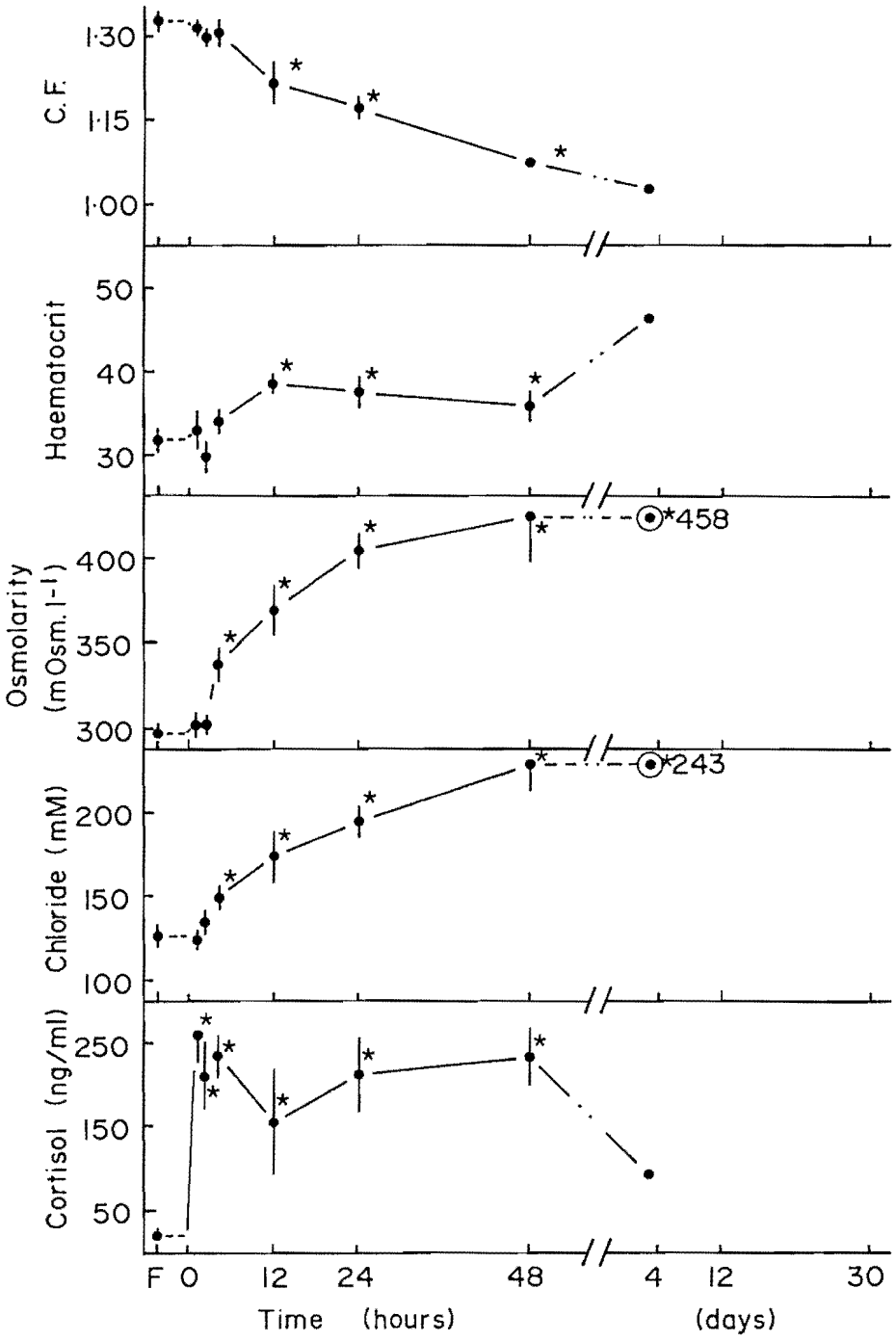

Figure 5. Physiological changes in sockeye salmon following an unsuccessful transfer to sea water in March 1986. F = initial freshwater sample. (O) freshwater controls. All values are means±S.E. Asterisks indicate a significant difference from initial freshwater sample ($P<0.05$).

Quinnat salmon

Successful Transfer

Quinnat salmon successfully adapted to sea water when transferred in July 1986. The physiological changes that occurred were similar to the changes that resulted when sockeye were transferred and successfully adapted to sea water (Figs. 3 and 6). Plasma cortisol concentrations rapidly rose in quinnat after seawater entry, increasing from pre-transfer levels of 72±13 ng ml^{-1}, to a recorded maximum after 2 hours of 193±27 ng ml^{-1} (a 2.7 fold increase, $p<0.01$). This was followed by a slower decrease to cortisol levels that were significantly lower than pre-transfer concentrations. The initial freshwater sample was high compared with the pre-transfer values of the January and March transfers.

When quinnat were introduced successfully into sea water, plasma osmolarity and chloride changed in a similar way to sockeye (Figs. 3 & 6). Both increased after transfer to reach a recorded maximum chloride concentration of 152±2 mmol l^{-1} and osmolarity of 358±5 mosmols l^{-1} after 24 hours. These levels had decreased and stabilised by 30 days to 140±2 mmol l^{-1} and 320±5 mosmols l^{-1}, but were still significantly higher than the freshwater-adapted quinnat values (both $p<0.01$).

There was no change in the haematocrit or condition factor of quinnat transferred into sea water (Fig. 6). Gill Na^+-K^+-ATPase activity did not change until 4 days post-transfer, after which it increased further to reach a recorded maximum of 15.04±2.91 µmol phosphate/mg protein/hr after 30 days (Fig. 6).

Partially Successful Transfer

Only some of the quinnat transferred into sea water in March successfully adapted. A brief but rapid 10.3-fold increase in plasma cortisol concentration occurred 1 hour after the seawater entry of quinnat (Fig. 7), which decreased to pre-transfer levels within 4 hours. A second rise in cortisol occurred after that time and it remained elevated until 12 days after the initial transfer. Large variation occurred in the 12 to 96 hours samples (Fig. 7).

The increases in plasma osmolarity and chloride concentrations after sea water transfer were greater and remained elevated longer than the changes that occurred during the successful July transfer of quinnat (Fig. 7). Chloride levels increased to a maximum recorded level of 164±11 mmol l^{-1} after 48 hours, and osmolarity to 385±12 mosmols l^{-1} after 24 hours. Between 12 and 30 days were

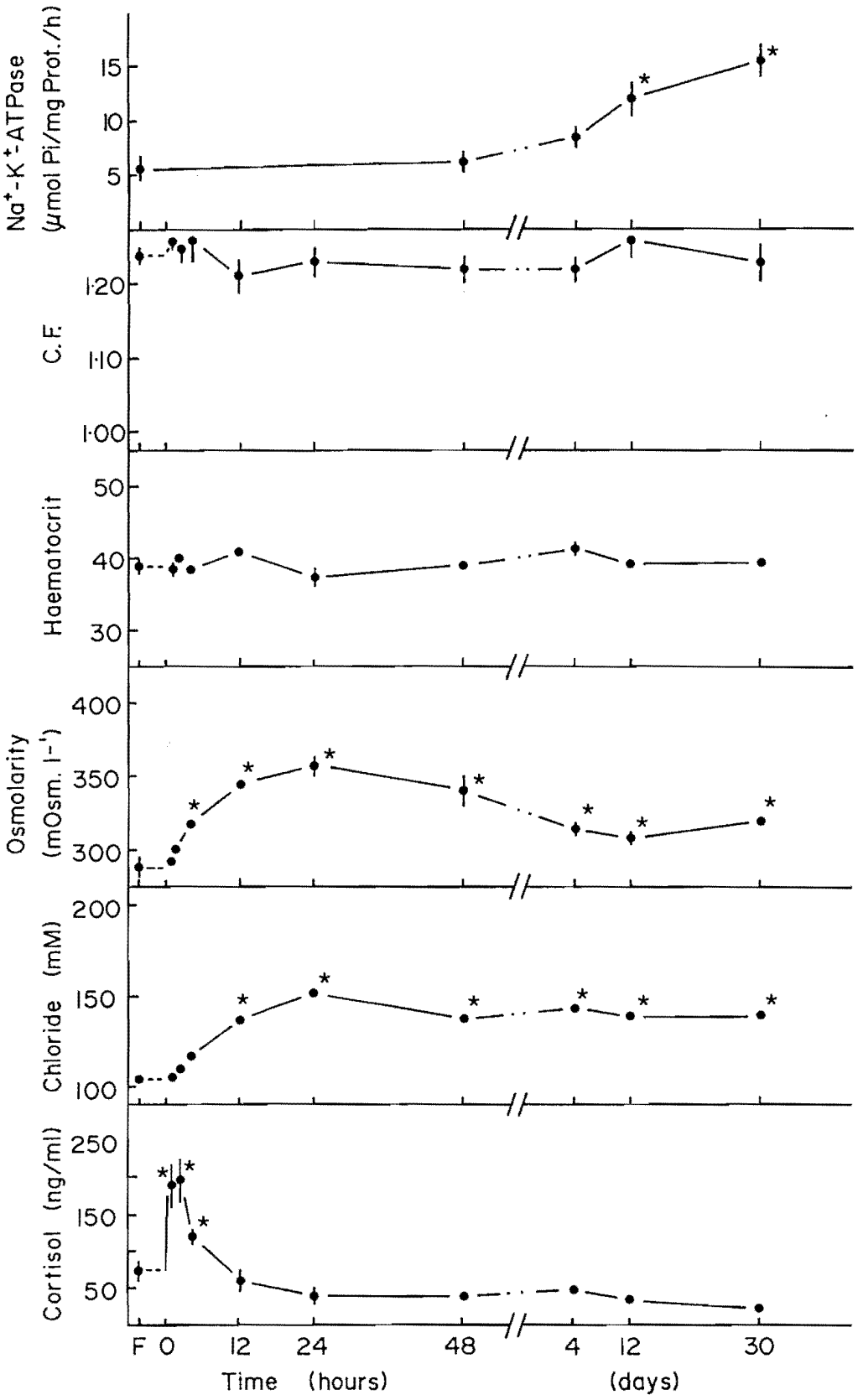

Figure 6. Physiological changes in quinnat salmon following a successful transfer to sea water in July 1986. F = initial freshwater sample. (O) freshwater controls. All values are means±S.E. Asterisks indicate a significant difference from initial freshwater sample ($P<0.05$).

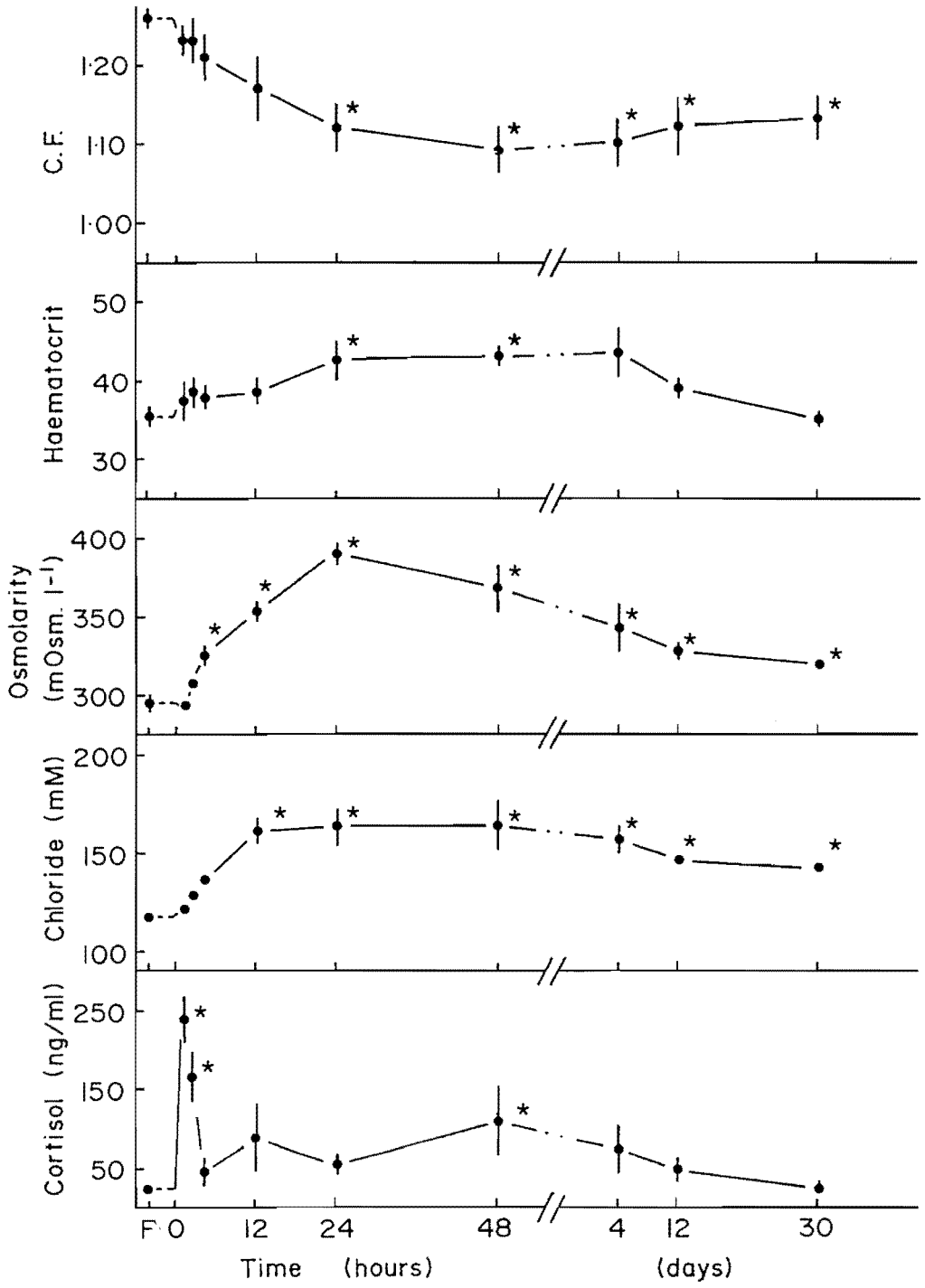

Figure 7. Physiological changes in quinnat salmon following a partially successful transfer to sea water in March 1986. F = initial freshwater sample. (O) freshwater controls. All values are means±S.E. Asterisks indicate a significant difference from initial freshwater sample ($P<0.05$).

required for these parameters to stabilise in the survivors, compared with 48-96 hours for the July transfer. Like the plasma cortisol concentrations of the same samples taken between 12 and 96 hours inclusive, there were comparatively large variations in chloride and osmolarity at these times.

There was a significant increase in haematocrit 12 hours after the introduction of quinnat into sea water (Fig. 7). The haematocrit increased further and remained elevated until 12 days post-transfer, after which it decreased to pre-transfer levels. The condition factor of the quinnat decreased during the first 24 hours post-transfer and remained at this level for the rest of the 30 days (Fig. 7).

Unsuccessful Transfer

Quinnat transferred in January failed to adjust to sea water and died within 5 days. Quinnat plasma cortisol concentrations increased 10.1-fold within the first hour of their seawater entry, but in contrast to the decrease observed between 1 and 4 hours during the July transfer, cortisol levels continued to increase further, reaching a recorded maximum of 255±47 ng ml^{-1} 48 hours post-transfer (Fig. 8). Cortisol concentrations were still significantly greater than the pre-transfer levels for the last sample at 4 days ($p<0.01$).

Plasma chloride concentrations and osmolarity increased after transfer to recorded maxima of 194±7 mmol l^{-1} at 48 hours and 409±14 mosmol l^{-1}, also at 48 hours post-transfer (Fig. 8). Compared with the successful July transfer of quinnat, there was a more rapid change, especially in osmolarity after this transfer. In addition, plasma chloride concentrations and osmolarity remained high and did not decrease substantially, nor stabilise to the levels found in the July transfer.

There was a significant increase in the haematocrit of quinnat 12 hours after transfer ($p<0.01$), which further increased to a recorded peak of 49.4±1.7% at 48 hours. By four days, the condition factor of quinnat decreased by 19.2%. This was much greater than the decrease in the condition factor in the March transferred fish (10.3%).

There was a small increase in gill Na^+-K^+-ATPase activity of quinnat 4 days post-transfer. This increase was considerably smaller than the increase in gill enzyme activity recorded after 4 days in July.

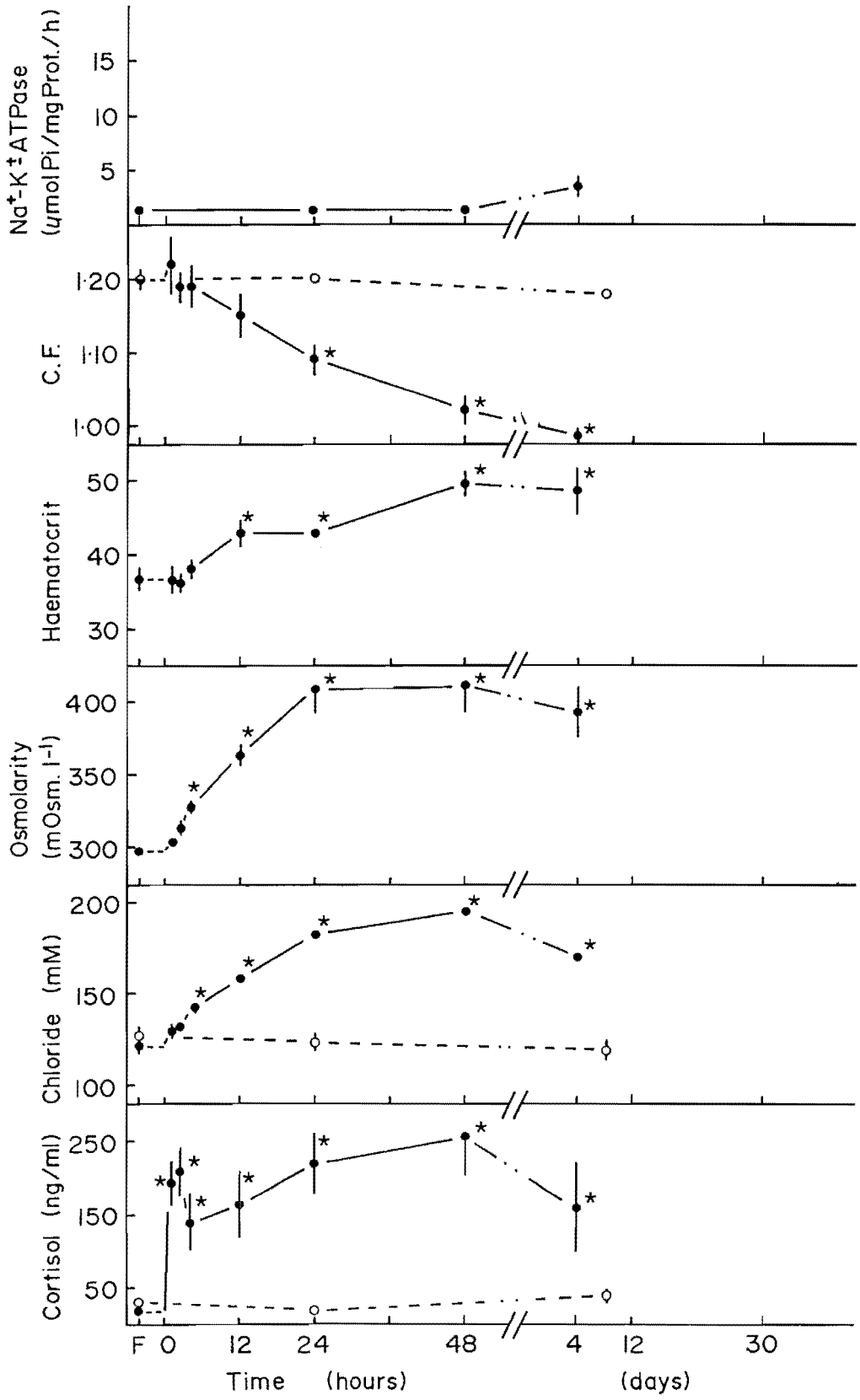

Figure 8. Physiological changes in quinnat salmon following an unsuccessful transfer to sea water in January 1986. F = initial freshwater sample. (O) freshwater controls. All values are means±S.E. Asterisks indicate a significant difference from initial freshwater sample ($P<0.05$).

Freshwater Controls

There were no significant changes in condition factor and plasma cortisol and chloride concentration in the quinnat that remained in fresh water at the same time as the January seawater transfer.

Discussion

Significant differences in the physiological changes of salmon transferred to sea water occurred between the salmon that successfully and unsuccessfully adapted to the new osmotic medium. There was, however, very little variation between the responses of quinnat and sockeye transferred to sea water. The two species of salmon showed similar physiological changes depending on whether they successfully or unsuccessfully adapted to sea water. The ability of sockeye and quinnat salmon to survive in sea water was affected by the method of transfer, by seawater temperatures, and the physiological (developmental) state of the fish (see Chapters 5 and 6).

The rate of mortality in the months where there was no survival resulted in a sigmoidally shaped survival curve. This shape is indicative of a normally distributed population of salmon with the rate of death related to the surface area to volume of the individual fish. Generally, the smaller fish, having a larger surface area to volume, died first and the larger salmon with the smaller surface area to volume died last. The smaller fish would suffer a more rapid osmotic imbalance than the larger fish due to the relatively greater surface area.

The cause of death in these fish appeared to be dehydration. There were large increases in osmolarity and plasma ion concentrations after seawater transfer that continued to increase until the death of the fish. They showed no ability to regulate the influx of ions and loss of water. The condition factor decreased markedly in these unsuccessful salmon and this reflects mainly the loss of water from the fish although a small part of the decrease could be due to the mobilisation of stored fuels.

Both quinnat and sockeye salmon successfully transferred to sea water during early winter (July 1986). By monitoring plasma ionic concentrations and osmolarity, it could be seen that ionic and osmotic homeostasis was achieved between 48 and 96 hours post-transfer. However, the achievement of

homeostasis did not coincide with increases in gill Na^+-K^+-ATPase activity levels. There were no significant increases in this enzyme until after four days post-transfer. In the Anguillidae and Salmonidae, high levels of gill Na^+-K^+-ATPase activity have been associated with seawater-adapted fish (Sargent *et al.*,1975; Folmar and Dickhoff, 1979; Chernitsky, 1980; Langdon and Thorpe, 1984). In this study, maximum levels of this gill enzyme did not appear to be a prerequisite for the salmon to achieve ionic and osmotic homeostasis, although relatively high levels of the enzyme were required in the smolts prior to transfer for successful adaptation (see Chapter 5).

Many other factors besides this one measure of an active transport mechanism (the gill ATPase) are working in the fish to maintain homeostasis. The ion transport mechanisms associated with the gut, kidney and bladder which were not studied also have a significant role in maintaining homeostasis and may explain why an increase in gill Na^+-K^+-ATPase did not coincide with the achievement of ionic and osmotic homeostasis.

Gill permeability changes with the transfer of fish into sea water. In freshwater-adapted fish, the gills consist of a tight epithelium which is highly impermeable to ion diffusion. In sea water, the gills have a relatively leaky epithelium which has a correspondingly greater permeability (Evans, 1984; Girard and Payan, 1984). The increases in gill Na^+-K^+-ATPase activity on seawater transfer may parallel the increases in gill permeability. Primmett *et al.*, (1988) found that increases in whole body permeability occurred during smoltification in Atlantic salmon (*Salmo salar*) which were resident in fresh water. Associated with the increases in permeability were increases in the gill Na^+-K^+-ATPase activity. It may be that the increases in this gill enzyme during sea water transfer may be stimulated by the increases in gill permeability.

The gradual rise in gill Na^+-K^+-ATPase levels after seawater transfer of euryhaline fish has been documented several times. Folmar and Dickoff (1979) found that a similar lag phase (with respect to ionic homeostasis) in the increase in gill Na^+-K^+-ATPase activity occurred in coho salmon (*O. kisutch*) after transfer to sea water. They suggested that temporary compensatory mechanisms (such as changes in chloride distribution and ion permeability) probably occur until the sodium pump mechanisms become fully operative.

Both the increase in gill permeability and increase in the Na^+-K^+-ATPase activity upon seawater transfer would require marked changes or modifications

of the gill epithelium at the cellular level. This would explain the relatively long time needed for a significant increase in this gill enzyme. Chloride cells located in the gill epithelium, and which are the site of high Na^+-K^+-ATPase activity, increase in size, complexity and number when euryhaline fish are transferred to sea water (Langdon and Thorpe, 1984; Karnaky, 1986; Pisam, 1987; see Chapter 7). These changes in the chloride cell morphology and distribution take longer to manifest than the 48-96 hours needed for the salmon to reach ionic and osmotic homeostasis. The rise in gill Na^+-K^+-ATPase activity is the result of two processes, an increase in the abundance of the chloride cells, and an increase in their content of this enzyme (Langdon and Thorpe, 1984).

There were no increases in gill Na^+-K^+-ATPase activity in unsuccessful salmon, which is not surprising, since the fish were dead by about the time increases in this enzyme were detected in the successfully transferred salmon. It should also be noted that the levels of enzyme activity were low in these salmon before they were transferred to sea water (compared with their successful counterparts), indicating that the salmon were not true smolts and so not physiologically prepared for the transition (see Chapter 5).

The abrupt transfer of salmon from fresh to sea water could be viewed as an effective stressor that has the ability to elicit a stress response from the fish. Typically down-stream migrating smolts spend some time in the boundary zone between the river and the ocean (in estuaries or around the river mouths) where there is a range of intermediate salinities. It is only in the farming of salmon, especially sea-cage rearing operations, where salmon are placed abruptly from fresh water to sea water. Matthews *et al.* (1986) suggested that the direct transfer of salmon into sea water could be used diagnostically to determine the relative health of the fish, using the fact that the transfer acts as a stressor. Unhealthy fish would die faster than the healthy fish. Following along the same lines, it would seem reasonable that a smaller stress response would occur in salmon that had undergone preparatory modifications (e.g. smoltification) before transfer into sea water. Conversely, salmon that were unprepared or not physiologically ready for seawater transfer would elicit a larger stress response. Barton *et al.* (1986) showed that the stress response is quantitative and cumulative and is dependent on the number of stressors and their magnitude.

There was little or no change in the haematocrit or the condition factor of sockeye and quinnat salmon that successfully adapted to sea water. An

increase in haematocrit of fish often indicates a stress response and this is especially noticeable in seawater-adapted fish (see Chapter 3). If this was used as a criterion of a stress response in fish, then it is reasonable to suggest that those salmon which successfully adapted to sea water were not stressed by the abrupt transition from fresh to sea water. An osmotic imbalance may also occur in fish stressed by a medium that is either hypo- or hyper-osmotic to their plasma (see Chapter 3). Although an osmotic imbalance occurred in the salmon transferred to sea water, this would be due mainly to the change in salinity and not as a consequence of the transfer acting as a stressor.

The rapid increase and subsequent return to basal levels of plasma cortisol after the direct and successful transfer of salmon to sea water is in accordance with other previous studies (Redding and Schreck, 1983; Redding *et al.*, 1984; Nichols and Weisbart, 1985; Patino *et al.*, 1987). Cortisol has a variety of effects in fish. It is known to influence the intermediary metabolism of the fish (a glucocorticoid role), and it also has been shown to play a part in the maintenance of ionic and osmotic homeostasis, thereby acting as a mineralocorticoid. It is commonly regarded as the putative seawater adaptation hormone. The rapid rise in plasma cortisol immediately after transfer could be interpreted in several ways. It could represent a stress response to the abrupt change in salinity, the hormone acting as a glucocorticoid, mobilising stored energy. Alternately or additionally it could be acting as a mineralocorticoid, having a more direct influence on the physiological adjustments needed for the salmon to survive in their new osmotic environment. The change in salinity should not only be viewed as a stressor with respect to a change in the external chemical composition. The initial rise in plasma cortisol may also have been stimulated by the increase in buoyancy associated with the salinity change.

In the salmon that were physiologically unprepared for a life in sea water and subsequently failed to adapt, the direct transfer of these fish to sea water would act as a severe stressor. Assuming this be true, then part of the physiological changes observed after transfer would comprise a stress response. As mentioned previously, an increase in haematocrit can constitute a stress response in fish. The haematocrit did increase in these unsuccessfully transferred fish, although it would be difficult to distinguish this increase as being caused directly by the change in external osmolarity or indirectly as part of a stress response. The haematocrit of fish can increase by a decrease in

plasma volume (haemoconcentration which could be associated with dehydration), by recruitment of red blood cells from the spleen, and/or by a swelling of the red blood cells, the latter relating more to a stress response. Catecholamines which are released into the blood stream if a fish is stressed have been shown to cause swelling of the red blood cells (DeVries and Ellory, 1981).

The same situation exists for all of the parameters studied, it being difficult to separate the physiological changes recorded into those constituting a stress response and those which are the direct result of the increase in osmolarity. A rise in plasma cortisol has been used in many studies as an indicator of a stress response (Pickering *et al.*, 1986; Sumpter *et al.*, 1986; Robertson *et al.*, 1987; see Chapter 3). In this study, plasma cortisol in the unsuccessful salmon rapidly increases after transfer and in contrast to the successful salmon, remained elevated. The initial, rapid rise in plasma cortisol was similar in successfully and unsuccessfully adapting salmon and these increases probably resulted from the same stimulus (e.g. it may have occurred as a response to an immediate increase in buoyancy of the fish on transfer to sea water). The continual high levels of plasma cortisol in the unsuccessful fish would more than likely result from the inability of the salmon to achieve ionic and osmotic homeostasis and/or could be associated with a general, lethal breakdown of homeostatic mechanisms within the fish (a pathological response).

CHAPTER 5

Seawater Adaptability of New Zealand's Sockeye (*Oncorhynchus nerka*) and Quinnat Salmon (*O. tshawytscha*): Physiological Correlates of Smoltification and Seawater Survival

Introduction

Differences have been shown to occur in the seawater adaptability of the various species of salmon, and also between populations or brood years of the same species (Zaugg, 1982). Interspecific and intraspecific differences in the hypoosmotic regulatory ability of salmon are further affected by environmental conditions such as water temperature and the seawater salinity, and also by the physiological state of the salmon. Prior to, or during, seaward migration juvenile anadromous salmon undergo a period of development which prepares them for their transition into sea water. This preparatory metamorphosis which involves extensive biochemical, physiological, morphological and behavioural changes is known as the parr-smolt transformation, or smoltification (see reviews by Folmar and Dickoff, 1980; Wedemeyer *et al.*, 1980; Barron, 1986; Hoar, 1988). Salmon have to reach a critical size and age before smoltification can occur. Once the critical size or age is reached, smoltification occurs in relation to environmental cues. Increasing photoperiod (spring), water temperature, flow-rate and the lunar cycle have all been shown to influence or initiate the parr-smolt transformation. The timing of the sea water transfer with respect to the developmental stage of the salmon is critical for high survival. For example, coho salmon transferred to sea water at the wrong time may either die or be severely stunted (Folmar *et al.*, 1982).

The parr-smolt transformation is characterised visually by the disappearance of vertical dark streaks that mark the sides of parr and by an increase in body silvering due to the deposition of guanine crystals in the epidermis (Markert and Vanstone, 1966; Gorbman *et al.*, 1982). Nevertheless, these changes in the salmonid's external appearance can not always be used as reliable indicators of the developmental stage of the salmon (pre-smoltified, smoltified or desmoltified). If salmon remain in fresh water after smoltifying they often revert back to a parr form (desmoltify) with respect to their osmoregulatory

status, but still retain the external morphology of a smolt for some time afterwards (Wedemeyer *et al.*, 1980). Because of the unreliability of using the external appearance of the salmon as an indicator of smoltification a variety of physiological parameters have been considered or used to predict the developmental stage of the salmon in fresh water. The seawater challenge test (Clarke and Blackburn, 1978; Blackburn and Clarke 1987) also provides a useful measure of the hypoosmotic regulatory status of salmon by measuring plasma ion concentrations (chiefly sodium) in salmon transferred to sea water for 24 hours.

Gill Na^+-K^+-ATPase activity levels have been widely used to monitor the osmoregulatory status of salmon. The greatest activity of this enzyme is localised along the baso-lateral membranes of the mitochondrial-rich chloride cells and is assumed to be coupled to one of the major ion pumps involved in maintaining osmotic and ionic homeostasis in fish. It is involved in both the excretion and uptake of sodium and chloride at the fish gills (Sargent *et al.*, 1980; Towle, 1981; De Renzis and Bornancin, 1984). An increase in gill Na^+-K^+-ATPase activity occurs when salmon are transferred to sea water (Sargent *et al.*, 1975; Chernitsky, 1980; see Chapter 4), which is reasonable, since in seawater fish ionic exchanges are larger compared with those observed in freshwater fish (Maetz, 1974; Maetz and Bornancin, 1975). A rise in gill Na^+-K^+-ATPase activity has also been associated with smoltification and an increase in the sea water capacity of salmon and this increase provides a useful predictive index (Zaugg and McLain, 1970; Hoar 1976; Folmar and Dickhoff, 1981; Ewing *et al.*, 1985).

The endocrine system has been implicated in the control of the parr-smolt transformation and hormones involved in this metamorphosis, such as thyroxine, tri-iodothyronine and cortisol, have been monitored in developing freshwater salmon (Folmar and Dickhoff, 1980; Specker and Schreck, 1982; Specker and Richman, 1984; Boeuf and Prunet, 1985; Dickhoff *et al.*, 1985; Young, 1986). Thyroxine and tri-iodothyronine have been shown to be relatively reliable indicators of smoltification (Grau *et al.*, 1985).

Quinnat (*Oncorhynchus tshawytscha*) and sockeye (*O. nerka*) salmon were introduced into New Zealand from North America in the late 1800's and early this century. The quinnat established an anadromous population; however sockeye, despite originating from a sea run population, became voluntarily land-locked and have reproduced and perpetuated in fresh water for the past 86 years. The biologies of salmon (coho and to a lesser extent quinnat and sockeye) have been

extensively studied in North America, and in recent years much of the research has concentrated on aspects of their aquaculture. An important area of study has been the sea water adaptability of salmon and hence their suitability for ocean ranching and sea cage rearing.

The chief purpose of this study was to investigate the seawater adaptability of New Zealand quinnat and sockeye salmon. Salmon (1985-1987 brood years, 6 to 18 months old) were subjected to rapid transfers from fresh water to sea water, being placed directly from fresh water into sea water with no intermediate salinities. Survival and growth in these seawater transferred salmon were monitored over 30 days. Various physiological parameters (gill Na^+-K^+-ATPase activity, cortisol, sodium, chloride, osmolarity, haematocrit and condition factor) were monitored in freshwater-adapted salmon and in salmon that had undergone a 24 hour seawater challenge test (Clark and Blackburn, 1978). The viability of these variables as indicators of sea water survival in sockeye and quinnat salmon is discussed.

Materials and Methods

Fish Stocks and Seawater Transfer Experiments

Quinnat and sockeye salmon were obtained from a Ministry of Agriculture and Fisheries hatchery on the Glenariffe stream, a tributary of the Rakaia river, and from a private pond rearing salmon farm at Prebbleton, Christchurch. Three brood years were sampled (1985-1987 broods) at periodic intervals between September 1985 to November 1987, and 0+ to 1 year old fish were used. Seawater transfers were performed at the Edward Percival Field Station, Kaikoura. Salmon were housed under a natural photoperiod and in 80 litre tanks at a density no greater than 6-10g fish weight per litre. Fresh water was displaced by sea water flowing in at a rate of 10-12 litres per minute and a salinity of 34‰ was achieved within 20 minutes. Control fish remained in fresh water at the same time as the sea water transfers were performed. The transfers, the water temperatures, and the weights and ages of the fish at the time of transfer are shown in Table 1.

Table 1. Water temperatures and the age and mean weights of fish transferred from fresh water into sea water.

Transfer Date	Species	Mean Weight (g) (x ± S.D.)	Age	Water Temp. (°C) F.W.	S.W.
1985 BROOD YEAR.					
Sept. 1985	sockeye	1.30±0.27	7	11.0	11.8
	quinnat	0.23±0.02	5		
Oct. 1985	sockeye	3.54±0.67	8	12.0	12.7
	quinnat	0.44±0.22	6		
Nov. 1985	sockeye	6.84±0.70	9	13.8	13.6
	quinnat	1.29±0.67	7		
Jan. 1986	sockeye	23.1±3.4	11	15.2	17.6
	quinnat	9.2±1.9	9		
Mar. 1986	sockeye	58.0±13.2	13	14.2	16.8
	quinnat	30.2±5.6	11		
May 1986	sockeye	73.4±8.6	15	13.8	14.2
	quinnat	49.4±10.1	13		
July 1986	sockeye	95.0±13.0	17	11.4	10.5
	quinnat	70.9±11.2	15		
1986 BROOD YEAR					
Nov. 1986	sockeye	2.23±0.95	9	14.5	16.3
	quinnat	1.03±0.72	7		
Dec. 1986	sockeye	4.0±1.8	10	17.0	17.0
	quinnat	1.94±1.24	8		
Jan. 1987	sockeye	8.1±2.4	11	17.0	18.8
	quinnat	8.2±2.2	9		
Feb. 1987	sockeye	21.2±1.6	12	17.2	19.0
	quinnat	18.5±4.0	10		
June 1987	sockeye	84.3±8.9	16	12.3	13.0
	quinnat	67.2±14.5	14		
1987 BROOD YEAR					
Oct. 5th 1987	quinnat	0.9±0.3	6		
Oct. 28th 1987	quinnat	3.0±1.4	7		
Nov. 1987	quinnat	7.0	8		

Following transfer, any dead fish were removed from the aquaria and the approximate time of death noted for each. The weights of the surviving fish were taken after 30 days to determine growth rates. The percentage of fish surviving after 30 days was taken as a relative measure of successful adaptation of the salmon to sea water.

Sampling

Fish from 1985 and 1986 brood years were sampled for physiological data. Freshwater adapted fish were sampled just prior to seawater transfer and for the 1985 transfers, salmon were also sampled 24 hours after seawater entry. A rapid and indiscriminate sample was achieved by catching 6 to 7 salmon with a hand net. The fish were immediately placed into an anaesthetic (2-phenoxyethanol, 20 mls mixed with 5 litres 30% sea water) that immobilised them within 30 seconds. Individual fish were measured and weighed, and blood extracted with heparinised (ammonium heparinate) syringes from the caudal vessels which were exposed by severing the caudal peduncle with a sharp scalpel. Once blood had been taken from the fish in a sample (approx. 8 minutes after netting), gill arches were exposed and excised. Observations were made of the degree of body silvering and the disappearance of parr marks.

Sample Analysis

Fish weight and length measurements were used to calculate Fulton's condition factor ($100W/l^3$, where W = weight in grams and l = length in centimetres). Blood samples were transferred to Eppendorf centrifuge tubes and centrifuged at 5000g for 3 minutes. Plasma was withdrawn with a pasteur pipette and frozen at -80°C in plastic vials. Plasma was subsequently analysed for cortisol concentrations, osmolarity, and sodium and chloride levels. Samples of whole blood were taken up into capillary tubes and spun at 20 000g for 3 minutes to determine the haematocrit.

Plasma cortisol concentrations were measured by radioimmunoassay (see Chapter 2) using duplicate 10µl samples. Osmolarities were determined from 8µl plasma samples with a vapour pressure osmometer. 5-10µl plasma samples were analysed with a Radiometer chloride meter to obtain chloride concentrations. Atomic emission spectrophotometry was used to measure the sodium concentration of 5ul plasma samples diluted with 5mls of distilled water.

The excised gill samples were washed in buffer (Na^+-K^+-ATPase homogenising buffer) and frozen at -80 °C in plastic vials until analysed for Na^+-K^+-ATPase

activity levels. These were measured by a modification of the methods used by Johnston *et al.* (1977) and Langdon *et al.* (1984) (see Chapter 2).

Statistical Analysis

Results are expressed as the mean standard error, or standard deviation. Statistical significance was determined by analysis of variance and Duncan's multiple range test or by the Student's t-test. Data that was not normally distributed was transformed before analysis.

Results

Seawater Survival and Seasonal Physiological Changes in Sockeye Salmon. 1985 Brood Year

There was high mortality (93%) when sockeye salmon were introduced into sea water in September 1985 (Fig. 1). The percentage of sockeye that survived seawater entry increased in the subsequent two transfers in October and November 1985, with nearly 100% survival in November. There was a corresponding increase in size of the salmon for these months with the salmon in November having a mean weight of 6.8±0.7g. Although of greater size, the sockeye salmon transferred in January and March 1986 failed to adapt to sea water (100% mortality for both transfers). The sea water temperatures were comparatively high (January =18.6 ℃, March = 15.8° C) for these transfers (Fig. 1). In May and July there was 100% survival of salmon introduced into sea water.

Overall no growth occurred in the sockeye that successfully adapted to sea water in September and October 1985. Most were stunted after 30 days in sea water, but a few of the remaining sockeye appeared to have grown though the overall growth rate for these two transfers reflected the larger number of stunted fish (Table 2). Significant growth did occur in the sockeye salmon transferred into sea water in November 1985, May and July 1986 (Table 2).

Freshwater Sockeye

Parr marks were clearly present on the juvenile sockeye in September and October. By November, the bands had disappeared and there was an increase in body silvering. Sockeye remained in this form throughout the rest of the experimental period (to July 1986).

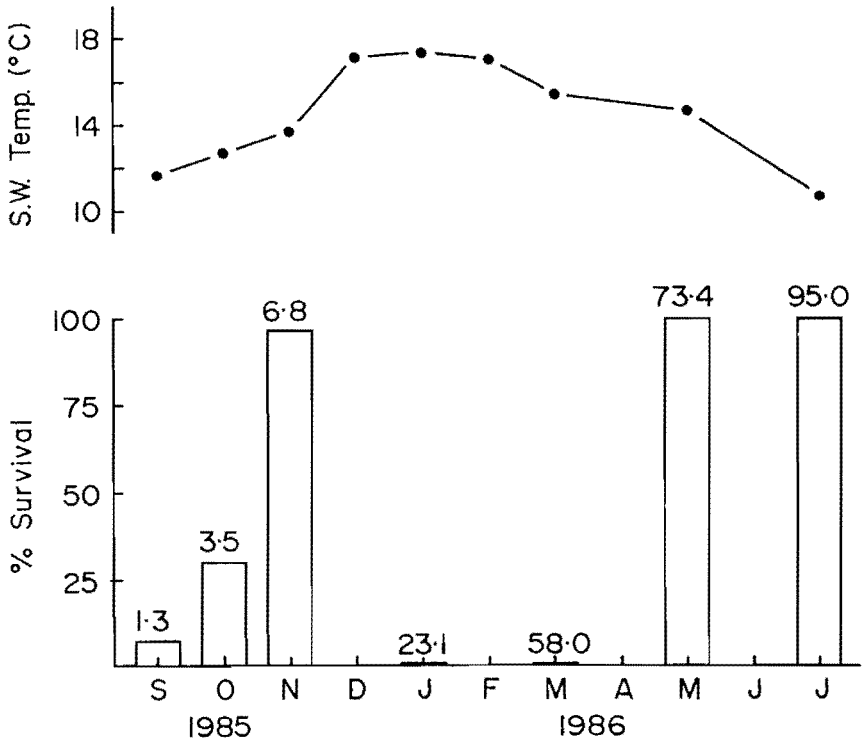

Figure 1. Survival of sockeye salmon (1985 brood year) 30 days after being rapidly transferred from fresh water to sea water. Mean wet weights of the sockeye are displayed above the histogram bars. Seawater (S.W.) temperatures are plotted on the top axis.

Table 2. Growth of salmon, 30 days after direct transfer from fresh water to sea water. * indicates significant growth ($P<0.05$).

	Initial Weight (g) (x ± S.D.)	Final Weight (g) (x ± S.D.)
SOCKEYE		
1985 BROOD		
September	1.30±0.27	1.4±0.7
October	3.54±0.67	3.0±0.3
November	6.84±0.70	8.9±0.8*
May	73.4±8.6	87.4±6.2*
July	95.0±13.0	160.1±33.8*
1986 BROOD		
June	84.3±8.9	103.6±10.8*
QUINNAT		
1985 BROOD		
Nov.	1.29±0.67	1.05±0.75
March	30.2±5.6	39.1±9.4
May	49.4±10.1	64.7±8.8*
July	70.9±11.2	87.7±4.8*
1986 BROOD		
February	18.5±4.0	27.1±5.7*
June	67.2±14.5	89.5±8.4*
1987 BROOD		
Oct. 28th	3.0±1.4	4.9±0.7
November	7.0	*

While still in fresh water, the gill Na^+-K^+-ATPase activities of sockeye salmon were significantly elevated in November 1985, and May and July 1986 ($p<0.01$, log transformed data) (Fig. 2). These elevations correlated well with the successful seawater transfers ($r=0.912$, $p<0.01$). A similar pattern occurred for plasma cortisol concentrations although the trends were not so clearly defined. Cortisol levels in November, May and July were not significantly different from the January sample but were higher than the rest of the monthly freshwater samples ($p<0.05$). There was also a significant correlation between seawater survival of sockeye and plasma cortisol levels ($r=0.775$, $p<0.05$).

Chloride concentrations of freshwater-adapted sockeye were negatively correlated with gill Na^+-K^+-ATPase activity ($r=-0.953$, $p<0.01$) and seawater survival ($r=-0.856$, $p<0.05$). Decreased chloride concentrations occurred when gill Na^+-K^+-ATPase activity levels were elevated (Fig. 2) and when seawater survival was high. No obvious trends existed for plasma sodium concentrations or osmolarity (Fig. 2). Haematocrit was, however, postively correlated with gill Na^+-K^+-ATPase activity ($r=0.891$, $p<0.05$) (Fig. 2). The condition factor of sockeye increased as the salmon grew in size until March 1986. In May and July, the condition factor decreased and levelled off (Fig. 2).

Seawater Sampled Sockeye

Greater differences existed between monthly samples when sockeye plasma was analysed 24 hours after seawater transfer (Fig. 3). In November, May and July, when sockeye successfully adapted to sea water, the plasma cortisol concentrations were significantly lower than the other monthly 24 hour samples ($p<0.01$). During the months when the sockeye failed to adapt to sea water, plasma cortisol levels were elevated 24 hours after seawater entry. Conversely, in the sockeye that successfully adapted to sea water, plasma cortisol levels were unchanged or lower than the freshwater values (see Figs. 2 and 3). A high negative correlation existed between the seawater survival of sockeye and the 24 hour plasma cortisol concentrations ($r=-0.912$, $p<0.01$). There was a reverse relationship between the plasma cortisol concentrations of sockeye sampled in freshwater and those sampled in sea water. If the cortisol concentrations were comparatively low in freshwater sockeye, then they tended to be comparatively high for their respective 24 hour seawater samples (see Figs. 2 and 3).

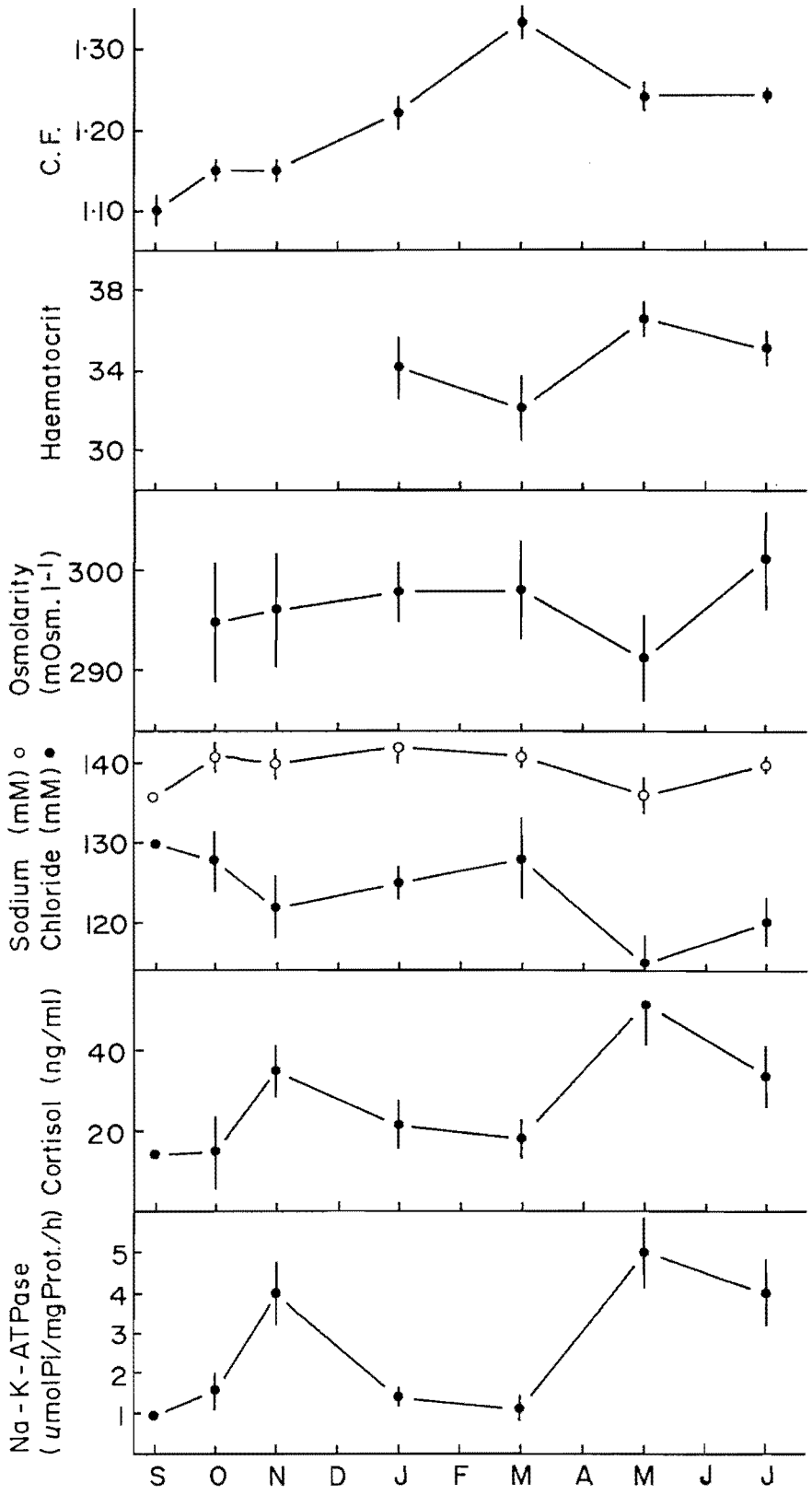

Figure 2. Variation in a range of physiological parameters (gill Na^+-K^+-ATPase activity, plasma cortisol, osmolarity, chloride and sodium concentrations, haematocrit and condition factor (C.F.) of sockeye salmon (1985 brood year) resident in fresh water. Each point represents the mean±S.E.

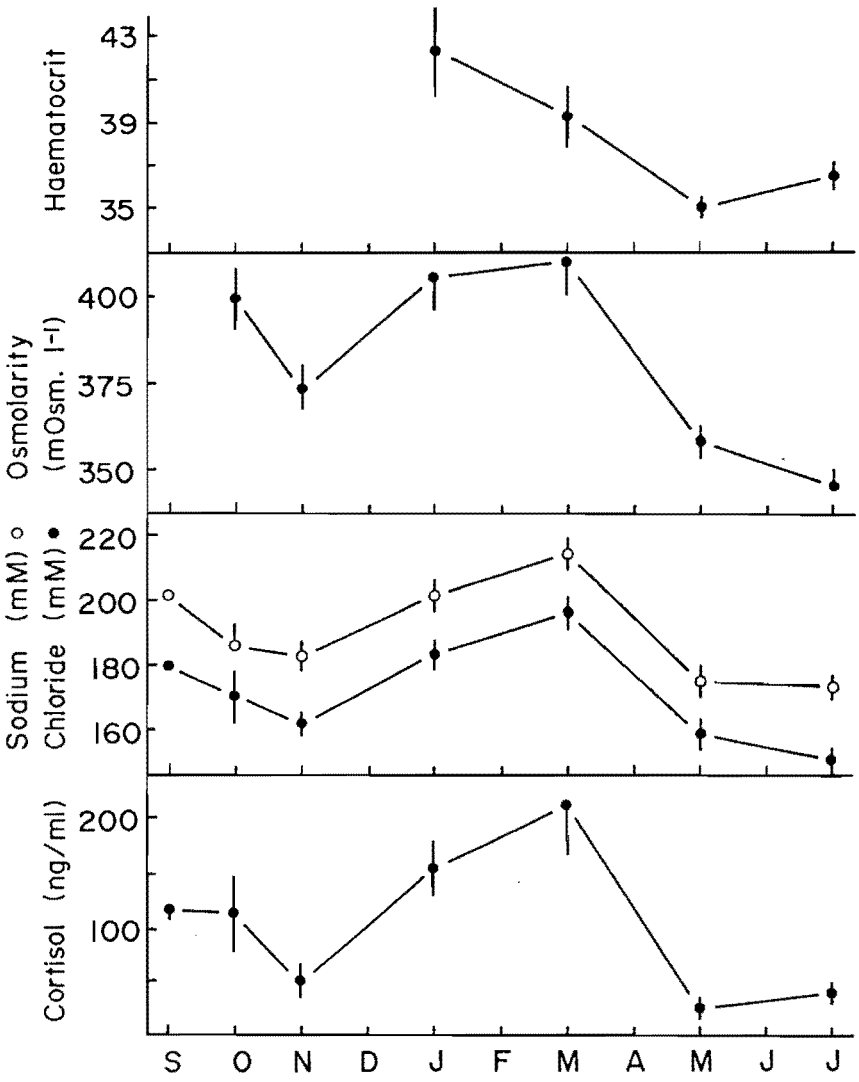

Figure 3. Variation in plasma parameters and haematocrit of sockeye salmon (1985 brood year) transferred into sea water and sampled after 24 hours. Each data point represents the mean±S.E.

Plasma sodium and chloride concentrations showed a similar trend to cortisol in sockeye sampled 24 hours post-transfer. Concentrations were significantly elevated in the salmon that failed to adapt to sea water with a peak occurring in March ($p<0.01$) (Fig. 3). Conversely, the lowest sodium and chloride concentrations occurred in salmon which transferred successfully into sea water. Gill Na^+-K^+-ATPase activity levels of freshwater-adapted sockeye were negatively correlated with the 24 hour sodium and chloride concentrations of the seawater-adapted sockeye (Na^+, $r=-0.924$, $p<0.01$; Cl^-, $r=-0.953$, $p<0.01$). These 24 hour post-transfer plasma ionic concentrations were also negatively correlated with the seawater survival of sockeye (Na^+, $r=-0.958$, $p<0.01$; Cl^-, $r=-0.956$, $p<0.01$). Plasma osmolarity in the 24 hour sampled sockeye showed very similar trends to sodium and chloride concentrations (Fig. 3). High osmolarities occurred in the unsuccessful (October, January and March) transfers and low osmolarities for the successful transfer (Fig. 3). Haematocrit was significantly

elevated in the salmon that did not adapt to sea water (January and March). Generally, the differences between the 24 hour monthly samples were more pronounced and correlated better with the seawater survival of sockeye than the changes in the monthly parameters that were taken prior to seawater transfer.

1986 Brood Year

Five seawater transfers were performed with the 1986 brood year sockeye. In November 1986, December 1986, January and February 1987, 100% mortality occurred after the sea water entry of sockeye, but in June 1987 sockeye adapted successfully (Fig. 4). The salmon transferred in June had grown by 30 days (Table 2). The sockeye of this brood year were considerably smaller than the 1985 brood year (Table 1).

Freshwater Sockeye

Parr marks were present on the sockeye until December 1986. By January 1987, the bands had disappeared and there was an increase in body silvering. The salmon retained this external morphology through to the final transfer in June 1987.

Gill Na^+-K^+-ATPase activity levels of freshwater sockeye were significantly lower at the times that the salmon failed to adapt to sea water (Nov. 1986, Dec. 1986, Jan. and Feb. 1987) compared with the enzyme activities of salmon in June 1987 ($p<0.01$) (Fig. 5). In contrast to the 1985 brood year changes in gill Na^+-K^+-ATPase activity levels were not correlated with the seawater survival of sockeye, although the enzyme activities were all below 3 µmoles phosphate mg $protein^{-1}$ hr^{-1} in the unsuccessful salmon. Like the 1985 brood year, the gill enzyme activities were negatively correlated with plasma chloride concentrations ($r=-0.945$, $p<0.05$). The chloride concentrations of freshwater-adapted sockeye were significantly lower in June when Na^+-K^+-ATPase activity was high than in the other monthly samples ($p<0.01$) (Fig. 5). The condition factor of sockeye increased from November 1986 to peak in February 1987 and then decreased slightly by June 1986 (Fig. 5).

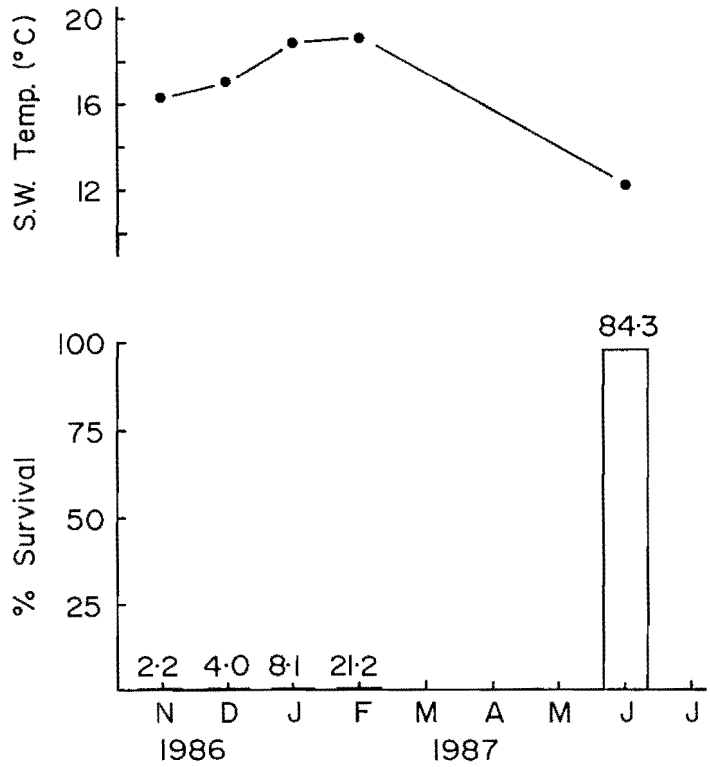

Figure 4. Survival of sockeye salmon (1986 brood year) 30 days after being rapidly transferred from fresh water to sea water. Mean wet weights of the sockeye are displayed above the histogram bars. Seawater (S.W.) temperatures are plotted on the top axis.

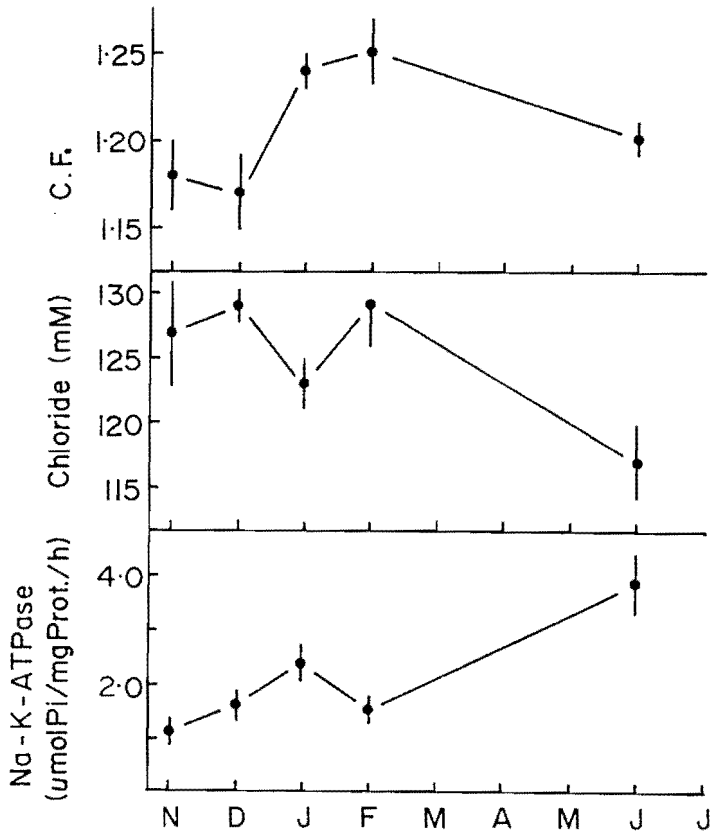

Figure 5. Variation in gill Na^+-K^+-ATPase activity, plasma chloride levels and condition factor (C.F.) of sockeye salmon (1986 brood year) resident in fresh water. Each data point represents the mean±S.E.

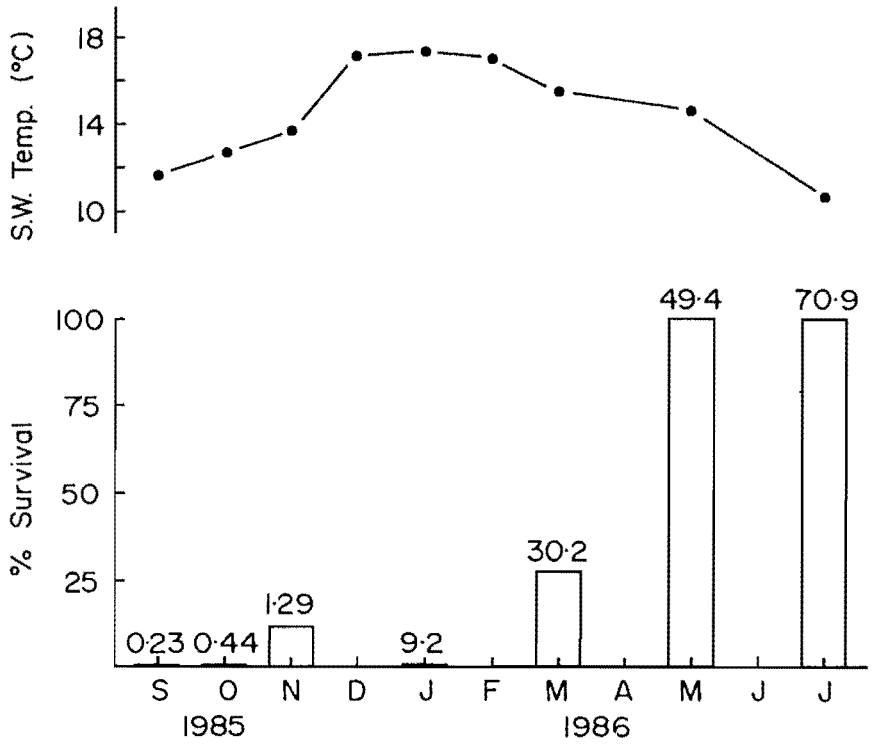

Figure 6. Survival of quinnat salmon (1985 brood year) 30 days after being rapidly transferred from fresh water to sea water. Mean wet weights of the sockeye are displayed above the histogram bars. Seawater (S.W.) temperatures are plotted on the top axis.

Seawater Survival and Seasonal Physiological Changes in Quinnat Salmon 1985 Brood Year

Quinnat salmon that were transferred directly into sea water in September and October 1985 failed to adapt (Fig. 6). In November 1985, 10.3% of the quinnat survived for the 30 days in sea water; however in January there was again 100% mortality. In March 1986, 27.3% of the quinnat transferred were alive after 30 days. Survival increased to 100% in May and July 1986. The quinnat that successfully transferred to sea water in November and March did not grow and were stunted, whereas in the May and July transfers the quinnat had significantly increased in weight after 30 days in sea water ($p<0.01$) (Table 2)

Freshwater Quinnat

The parr marks of juvenile quinnat salmon had partially disappeared by November 1985 and were completely absent in January 1986, with a corresponding increase in body silvering. The quinnat retained this external morphology for the rest of the transfers.

As the quinnat salmon increased in size, there was a corresponding increase in the gill Na^+-K^+-ATPase activity (Fig. 7) a maximum being recorded in July 1986.

There was a positive correlation between the percentage of quinnat surviving sea water transfer and gill Na^+-K^+-ATPase activity: the higher the enzyme activity the greater the survival ($r=0.916$, $p<0.05$). Plasma cortisol concentrations were significantly greater in the freshwater quinnat groups that successfully adapted to sea water ($p<0.01$). A very high, positive correlation existed between cortisol concentration and gill Na^+-K^+-ATPase activity ($r=0.940$ $p<0.05$). Plasma chloride concentrations decreased over the months in freshwater-adapted quinnat and were negatively correlated with gill Na^+-K^+-ATPase activity (ATPase, $r=-0.936$, $p<0.05$). Osmolarity showed a similar trend to chloride concentrations, decreasing with increasing age of the quinnat but was not correlated with seawater survival, despite a significant difference between the January and July samples ($p<0.05$). The haematocrit of freshwater-adapted quinnat showed no obvious trends. There was an increase in condition factor of quinnat between September 1985 and March 1986. The condition factor rose to a peak in March 1986 and remained at this level through May and July 1986 (Fig. 7).

Seawater Sampled Quinnat

Compared with the plasma cortisol concentrations of the freshwater-adapted quinnat the opposite trend occurred in cortisol levels of the quinnat sampled 24 hours after seawater entry (Fig. 8). At 24 hours, significantly lower cortisol concentrations occurred in the quinnat that successfully adapted to seawater compared with the unsuccessful transfers ($p<0.01$). A maximum mean cortisol concentration of 222 ± 39 ng ml^{-1} was recorded in January 1986 when the quinnat failed to adapt to sea water. There was a decrease with age in the chloride concentrations of the seawater transferred salmon sampled after 24 hours. The lowest chloride concentrations were recorded in May and July 1986 and chloride levels were negatively correlated with seawater survival ($r=-0.879$, $p<0.05$). The chloride levels at 24 hours post-transfer were higher than the pre-transfer concentrations. The changes in the osmolarity of the 24 hour seawater quinnat were also similar to the changes that occurred in the freshwater-adapted quinnat, the lowest osmolarities occurring in quinnat that successfully adapted to sea water in May and July 1986. Similarly for the haematocrit of quinnat sampled 24 hours after transfer, it was significantly lower in the quinnat that adapted to sea water ($p<0.01$) (Fig. 8).

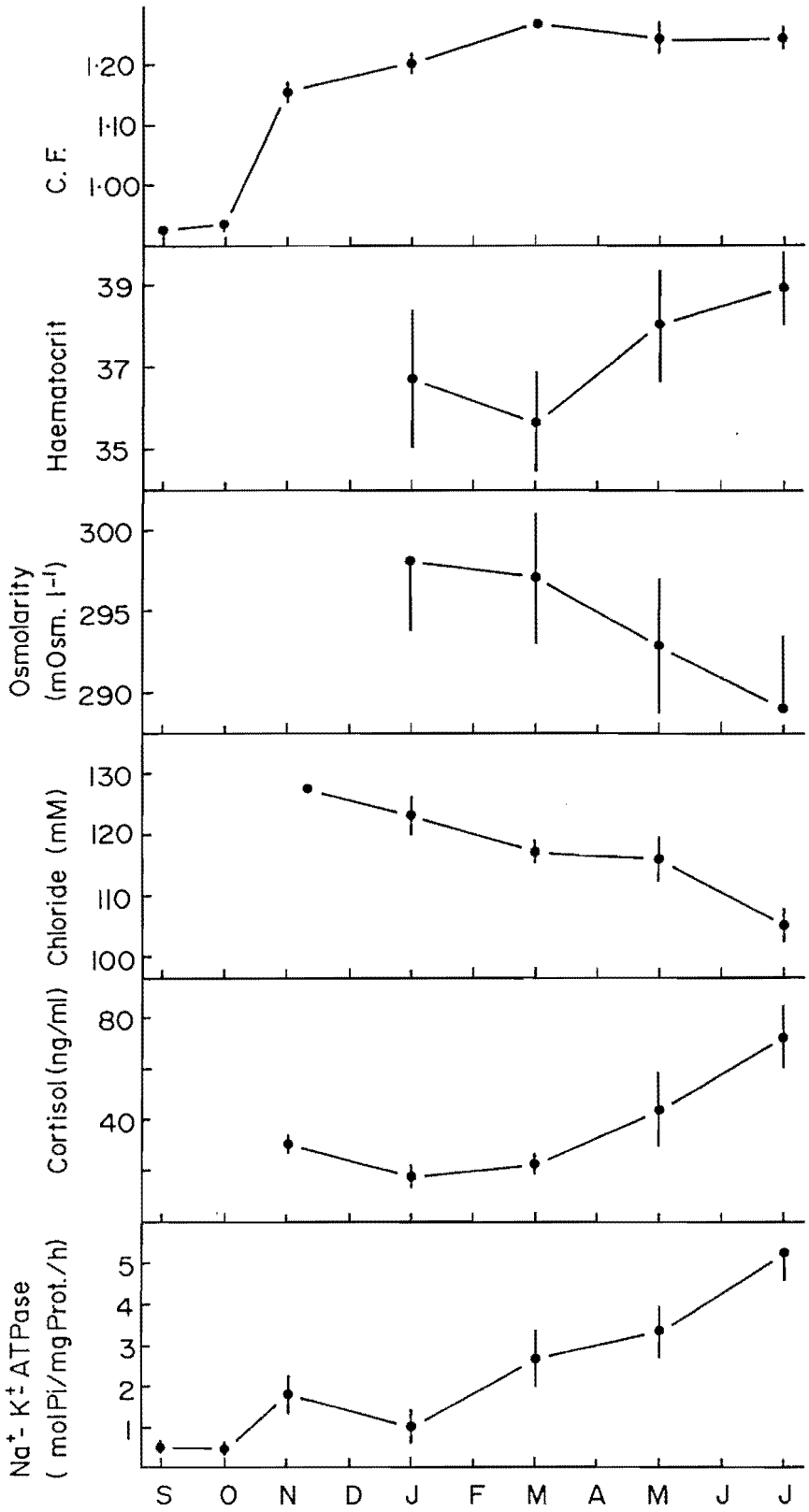

Figure 7. Variation in a range of physiological parameters (gill Na^+-K^+-ATPase activity, plasma cortisol, osmolarity, chloride concentrations, haematocrit and condition factor (C.F.) of quinnat salmon (1985 brood year) resident in fresh water. Each point represents the mean±S.E.

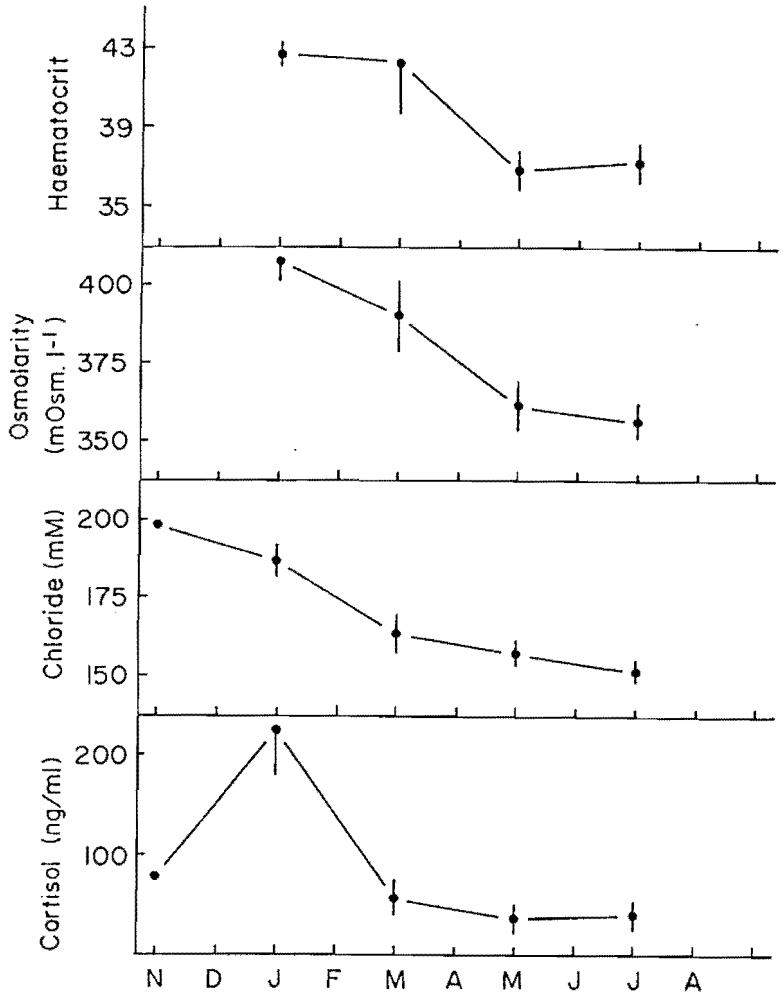

Figure 8. Variation in plasma parameters and haematocrit of quinnat salmon (1985 brood year) transferred into sea water and sampled after 24 hours. Each data point represents the mean±S.E.

1986 Brood Year

Quinnat salmon of this brood year were smaller than the 1985 brood. Quinnat did not successfully adapt (100% mortality) when transferred into sea water in November 1986, December 1986, and January 1987 (Fig. 9). In February 1987, 18.5% of the quinnat transferred to sea water were alive after 30 days and these salmon had grown significantly over this period (Table 2). Quinnat did adapt successfully to sea water in July 1987 and significant growth had occurred after 30 days (Table 2).

Freshwater Quinnat

Parr marks were present on quinnat through to February 1987. By July 1987 the parr marks had completely disappeared and the fish had taken on a very silvery external appearance.

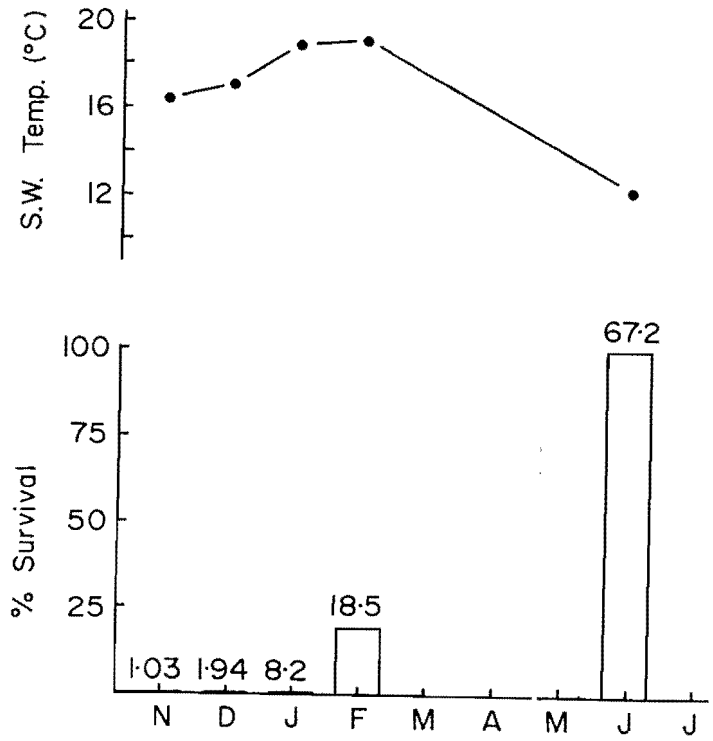

Figure 9. Survival of quinnat salmon (1986 brood year) 30 days after being rapidly transferred from fresh water to sea water. Mean wet weights of the sockeye are displayed above the histogram bars. Seawater (S.W.) temperatures are plotted on the top axis.

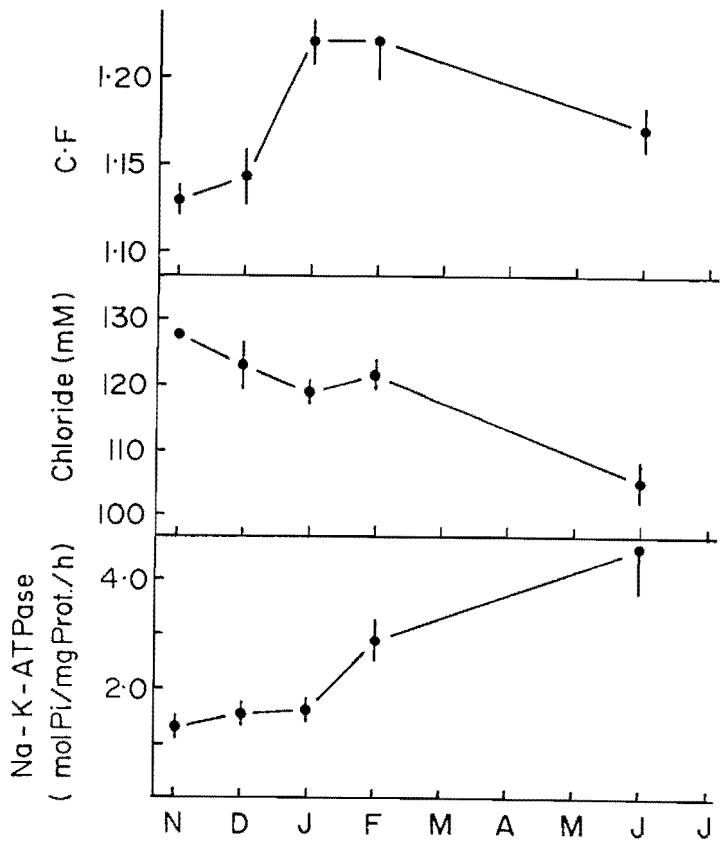

Figure 10. Variation in gill Na^+-K^+-ATPase activity, plasma chloride levels and condition factor (C.F.) of quinnat salmon (1986 brood year) resident in fresh water. Each data point represents the mean±S.E.

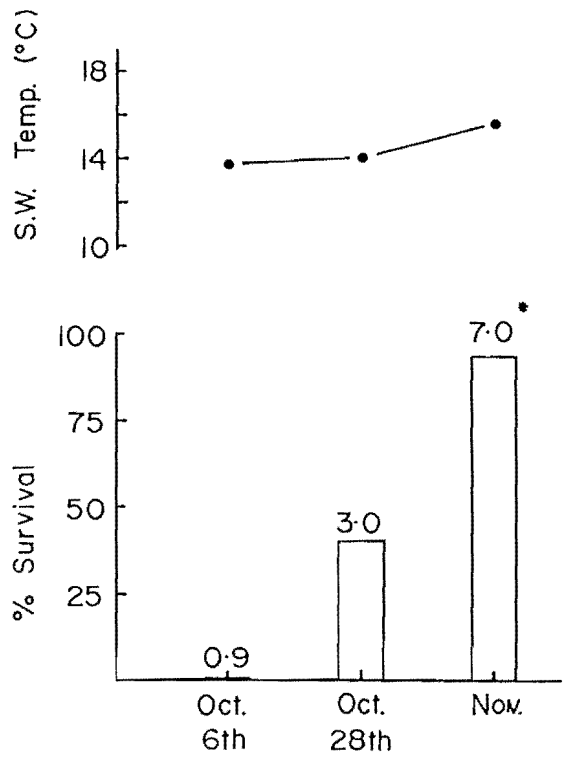

Figure 11. Survival of quinnat salmon (1987 brood year) 30 days after being rapidly transferred from fresh water to sea water. Mean wet weights of the sockeye are displayed above the histogram bars. Seawater (S.W.) temperatures are plotted on the top axis. (* - survival data estimated from sea-cage rearing operation).

Gill Na^+-K^+-ATPase activity levels of freshwater-adapted quinnat were significantly higher in February and June than for the other monthly samples ($p<0.01$) (Fig. 10). The enzyme activity was highly positively correlated with the survival of quinnat transferred to sea water ($r=0.994$ $p<0.01$). Chloride concentrations were significantly lower in the quinnat that successfully adapted to sea water in June 1986 and were negatively correlated with both sea water survival ($r=-0.903$, $p<0.05$) and gill Na^+-K^+-ATPase activities ($r=-0.943$, $p<0.05$) (Fig. 10). There was an increase in the condition factor of quinnat that peaked in January - February. The condition factor of the June quinnat was lower than in January and February ($p<0.05$).

1987 Brood

The quinnat of this brood year were considerably larger than the quinnat of 1985 and 1986 brood years measured at similar ages. No quinnat survived the initial transfer to sea water in early October 1987. There was an increase in survival of the quinnat transferred into sea water 3 weeks later and a further increase in survival for the last sea water transfer (Fig. 11). The weight of the fish for the last transfer was 7.0g.

Parr marks were clearly visible on the quinnat transferred in early October 1987, but three weeks later the bands had all but disappeared and the quinnat had a silvery external morphology.

Sockeye and Quinnat Freshwater Controls

The sockeye and quinnat that remained in fresh water during the sea water trial periods suffered no mortality.

Discussion

New Zealand sockeye salmon (*O. nerka*) were able to successfully adapt and grow in sea water. Eighty six years of being landlocked and excluded from sea water has not destroyed the ability of these salmon to successfully make the transition from fresh water to sea water. Unfortunately there are no data on the sea water capacity of New Zealand sockeye prior to this study, so whether the ability of sockeye to adapt to sea water has been reduced because of their isolation from sea water is unknown. Presumably, there has been no change in the sea water capacity of these sockeye as they show a similar pattern of seawater tolerance to the North American Pacific salmon. Burton and Idler (1984) found that Newfoundland landlocked Atlantic salmon (*Salmo salar*) had the capacity to adapt to sea water but only 11% of the fish tested survived the transition into sea water. Depending on the time of the transfer, between 0% and 100% survival was recorded for sockeye after being in sea water for thirty days. As expected, New Zealand's quinnat salmon (*O. tshawytscha*) were able to adapt to sea water.

The ability of salmon to survive in sea water does not necessarily indicate that the salmon have successfully adapted to the sea water. Growth of salmon after transfer provides a better indicator of success. In several transfers the salmon failed to grow after entry to sea water and were stunted. These salmon appeared to be able to tolerate the marine environment but were unable to grow. It would appear that all of the energy that would normally used for muscle growth was used instead to maintain osmotic and ionic homeostasis. This lack of growth was found to occur in coho salmon (*Oncorhynchus kisutch*) that were prematurely transferred to sea water (Clarke and Nagahama, 1977). More recently, Folmar *et al.* (1982) found that if coho salmon were transferred at an inappropriate time to sea water the fish developed abnormally resulting in two forms of seawater-adapted parr which they termed the 'stunt' and the 'parr-revertant'. They suggested that the stunts arise from premature transfers to sea water while parr-revertants occur when salmon are transferred to sea water during desmoltification. The stunted salmon in this study were not separated into 'stunts' or 'parr-revertants'. All of the stunted

salmon had a similar external morphology, characteristically darkly pigmented and emaciated. Stunting usually occurred in the salmon that were transferred to sea water with low Na^+-K^+-ATPase activities (that is, not smolted).

Generally there is an increase in the hypo-osmotic regulatory ability of salmon as they grow older and larger (Conte and Wagner, 1965; Wagner *et al.*, 1969). This can be related, in part, to a decrease in the surface area to volume of the larger fish. A critical minimum size needs to be attained before the salmon can successfully make the transition into the marine environment and this size generally equates to the minimum size needed before the parr-smolt transformation can be activated. New Zealand sockeye needed to be over 6.0g and quinnat greater than 7.0 g to adapt successfully to sea water. Clarke (1982) gave values of body weight for North American sockeye and quinnat which showed optimal hypoosmoregulatory capacity of 2g and 5g, respectively. In this study, some of the sockeye were able to go into sea water at a weight of 2g but most of these fish were stunted and showed poor hypoosmoregulatory ability. Clarke and Shelbourne (1985) found that some populations of North American quinnat were able to regulate in sea water at the fry stage. This was not the case for the New Zealand quinnat.

In this study sockeye and quinnat were able to adapt successfully to the marine environment primarily in spring (depending on the size of the fish) and/or during early winter. In North America, it is usual for salmon to smoltify and to adapt successfully to sea water in spring and during autumn (Buckman and Ewing, 1982; Hoar 1988). The New Zealand sockeye and quinnat were able to smoltify and to go into sea water as underyearlings, a feature that is not uncommon for the quinnat in North America; however, sockeye rarely smoltify in their first year in North America and normally undergo their metamorphosis as yearlings or 2 year olds. The growth rates of sockeye are considerably faster in New Zealand compared with their Northern Hemisphere counterparts due to higher freshwater temperatures. These higher temperatures would have an effect not only on growth but could also influence the hypoosmotic regulatory development of the salmon (smoltification).

Together with increasing photoperiod, rising temperatures like those occurring during spring help to initiate the parr-smolt transformation. By increasing the freshwater temperature smolting has been shown to occur sooner. However, too high a temperature can have an inhibitory effect on smoltification and can accelerate desmoltification so that the time that the salmon is in a smoltified

state is short. Zaugg (1981) found that freshwater temperatures greater than 13° C generally abolished the rise in gill Na^+-K^+-ATPase activity of steelhead trout. Hoar (1988) suggests an optimum temperature for the parr-smolt transformation of 10-12° C. The sockeye and quinnat in this study were reared at freshwater temperatures well in excess of this suggested optimum. In New Zealand during the summer months, freshwater temperatures are often in excess of 17°C. The effect of such temperatures on the parr-smolt transformation of quinnat and sockeye was not directly examined in this study, although the high temperatures may partially account for the delayed rise in gill Na^+-K^+-ATPase activity until winter when freshwater temperatures were cooler. In the brood years in which a significant smolt size had not been reached by spring, little or no increases in gill Na^+-K^+-ATPase activity were recorded during the summer. It can therefore be assumed that smoltification was inhibited over the summer months and early autumn by the high water temperatures.

Gill Na^+-K^+-ATPase activities have been used frequently to determine the stage of development in anadromous salmon. This enzyme is located at and concentrated along the baso-lateral membrane of chloride cells which are found at high densities in the gill epithelium. A peak in gill Na^+-K^+-ATPase activity levels during smoltification was first reported by Zaugg and McLain (1970) for coho salmon. Since then, comparable increases in gill Na^+-K^+-ATPase activity during the parr-smolt transformation have been reported for most of the anadromous salmonid species. Boeuf and Harache (1982) found that non anadromous species such as strains of brown (*Salmo trutta*) and rainbow trout (*Salmo gairdneri*) do not smoltify and hence do not show seasonal increases in gill Na^+-K^+-ATPase activities. Although non-anadromous, the New Zealand sockeye still exhibits visual changes that correspond to smoltification and this is further supported by coincidental increases in gill Na^+-K^+-ATPase activity. However, New Zealand's sockeye originally came from an anadromous population and this feature probably explains why the salmon exhibit these rhythms in enzyme activity which are normally associated with anadromy.

Plasma cortisol and ionic concentrations (especially chloride) in freshwater salmon were highly correlated with gill Na^+-K^+-ATPase activities. When enzyme activities were high there were correspondingly high cortisol levels and low chloride concentrations. These changes could be explained in several ways. Increases in cortisol may indicate that it has a controlling or regulative function in smoltification, and/or the increases may be the result of a generalised stress response to the transformation. Cortisol has a variety of

effects influencing the ionic balance of the fish (mineralocorticoid effect) as well as an effect on the intermediary metabolism (glucocorticoid) (Chester-Jones *et al.*, 1969; Chakraborti *et al.*, 1987).

The control of smoltification undoubtedly involves the endocrine system of which the interrenal gland more than likely plays a role. The thyroid gland has also been implicated. Springtime elevations in plasma concentrations of thyroid hormone of anadromous salmonids coincide with the period of the parr-smolt transformation and possibly play a role similar to that in amphibian metamorphosis. There have been several studies also documenting a rise in plasma cortisol during smoltification (Specker and Schreck, 1982; Langhorne and Simpson, 1986; Richmann and Zaugg, 1987). Histological studies have shown that the interrenal cells undergo hypertrophy during smoltification (Olivereau, 1962; Nishioka *et al.*, 1982). The cells producing adrenocorticotropic hormone (ACTH) in the pituitary have also been found to be more active during smoltification (Nishioka *et al.*, 1982). From results in this study, plasma cortisol concentration increased at the same time as Na^+-K^+-ATPase activity rose which could suggest a cause and effect relationship between these two variables. Cortisol may be stimulating the increase in gill Na^+-K^+-ATPase activity, although Langdon *et al.* (1984) found that cortisol administered to Atlantic salmon (*Salmo salar*) did not stimulate an increase in this gill enzyme although ACTH did have an effect.

While in fresh water, salmon undergo preparatory modifications during smoltification that allow them to survive in the marine environment. Once salmon are in sea water, their freshwater ionic and osmotic regulatory mechanisms have to be inhibited and their salt water regulatory mechanisms quickly activated. These changes include changes in permeability and ion extrusion/absorption processes. Freshwater-adapted fish are more impermeable than seawater-adapted fish (Evans 1984). Smoltification allows the reversal of osmoregulatory mechanisms to be completed successfully and suggests that the salmon's osmoregulatory mechanisms are partially modified for the marine environment while the fish is still resident in fresh water. If this is the case then an ionic and osmotic imbalance would be likely. This was observed in the freshwater-adapted quinnat and sockeye that subsequently successfully adapted to sea water (presumed to be in a smoltified state). Primmett *et al.* (1988) found that whole body permeability changed in smolting Atlantic salmon. A net loss of sodium occurred slightly after maximal gill Na^+-K^+-ATPase activity and preceded maximal seawater tolerance. These changes in permeability could

explain, in part, the low plasma chloride concentrations of salmon with high gill Na^+-K^+-ATPase activities.

The seawater preparatory changes that occur in the freshwater-adapted salmon during smoltification could act as a stressor, eliciting a stress response in salmon. Part of the generalised stress response of fish is an increase in plasma cortisol concentration (Mazeaud and Mazeaud, 1981; see Chapter 3). An osmotic imbalance also results when fish are stressed. Freshwater fish tend to lose ions and gain water osmotically, whereas in marine fish there is an influx of ions and water is lost. Therefore, the osmotic imbalance resulting from stress might explain the decrease in plasma ions or augment the loss resulting directly from the preparatory modifications. It should be noted that this decrease in ionic concentrations may be the stressor and therefore cause the increase in plasma cortisol. Supporting the assumption that the fish are stressed was an elevated haematocrit that coincided with high gill Na^+-K^+-ATPase levels. Increased haematocrit is often associated with a stress response in fish due in part to the swelling of the red blood cells from circulating catecholamines (DeVries and Ellory, 1981).

When changes that occurred in the 24 hours following transfer are compared between monthly samples, a clearer picture of the hypoosmoregulatory ability of the salmon emerges. Plasma osmolarity and ionic concentrations indicate whether the salmon are able to regain homeostasis within 24 hours of seawater transfer. For example, when chloride concentrations were greater than 170 mmol l^{-1}, salmon generally failed to make the transition into sea water as they eventually became dehydrated and died; that is, high chloride concentrations indicated osmoregulatory failure (see Chapter 4 for details). In contrast to what happened in freshwater, cortisol concentrations were elevated in salmon that failed to go into sea water and which had correspondingly low gill Na^+-K^+-ATPase activities while in fresh water. The increases in plasma cortisol 24 hours after seawater transfer may be the reaction of a generalised stress response (chiefly a glucocorticoid response), and/or the elevated cortisol may be secreted to restore osmotic homeostasis by attempting to stimulate hypoosmotic mechanisms such as increasing chloride cell ion pumps, in particular Na^+-K^+-ATPase (a mineralocorticoid response). High haematocrits in these salmon also suggest that the salmon with elevated plasma cortisol concentrations were stressed.

Physiological Indices of Seawater Survival of Salmon.

Fish size is too simplistic to be used as an effective indicator of likely sea water survival. It is useful to know the minimum size needed for the salmon to be considered for seawater transfer. Once this minimum size is attained, the seawater adaptability of the salmon is influenced by environmental conditions which in turn have an effect on the timing of smoltification. A more accurate measure is needed of the physiological status of the salmon. Size was found to give no real indication of the fish's osmoregulatory ability.

Changes in external morphology (disappearance of parr marks and increases in body silvering) and condition factor of quinnat and sockeye salmon have been used more effectively to time the transfer into sea water. There is a reduction in the condition factor around smoltification, mainly due to the loss of lipid from the body wall external to the myotome (Sheridan *et al.*, 1983, 1985a, 1985b; Sweeting *et al.*, 1985). Occurring at about the same time as the decrease in condition factor is a disappearance of parr marks and an increase in body silvering. Changes in condition factor of sockeye and quinnat were small so the sensitivity of this variable as an indicator is low. Both the changes in condition factor and in the extent of body silvering were crude measures of smoltification in New Zealand's hatchery reared quinnat and sockeye salmon. Both of these indices are unreliable if the salmon desmoltify, as neither return back to the conditions that were representative of parr. Desmoltified sockeye and quinnat still had the visual appearance and condition factor of smolts.

Gorbman *et al.* (1982) found that in addition to the morphological changes in pigmentation there were a variety of other external changes that coincided with smoltification in coho salmon, *Oncorhynchus kisutch*. These include emergence and growth of teeth on the maxilla, mandible and tongue, changes in the pelvic fin and folds adjacent to the cloacal opening and variation in the growth of the scales.

In this study, gill Na^+-K^+-ATPase activity was found to be a good indicator of sea water survival in sockeye and quinnat salmon. When there were elevated levels of this enzyme, there was generally high seawater survival in sockeye and quinnat. The usefulness of this enzyme as an indicator is limited by environmental conditions. Seawater temperature has an overriding effect, so even if enzyme levels are high, sea water temperatures greater than 17-18°C reduce the survival rate of transferred salmon (see Chapter 6 for details).

Sea water temperatures which salmon are exposed to in New Zealand are generally higher than those encountered in North America: especially in the coastal areas used for sea cage rearing where water temperatures may reach 19-20°C.

Of all the variables measured in fresh water, gill Na^+-K^+-ATPase activity levels proved to be the most reliable indicator of sea water survival. However, Ewing *et al.* (1985) found that as an indicator for the timing of release of salmon from ocean ranching operations, gill Na^+-K^+-ATPase activities of juvenile coho and quinnat salmon were not a good predictor of high adult returns. They suggest that enzyme activity levels can be affected by a multitude of environmental and rearing procedures and that the levels measured at the hatcheries may change before the salmon reach the ocean. This would be more likely to occur in salmon that have been released from a hatchery that is situated far from the ocean. The ocean ranching operations that are situated on the coast may have a higher degree of success as the salmon are released almost straight into the marine environment and are capable of withstanding abrupt transfer.

Several other physiological parameters have been suggested as indices of smoltification and successful seawater adaptation. The thyroid gland has been shown to be activated during the parr-smoltification (Boeuf and Prunet, 1985; Virtanen and Soivio, 1985; Young, 1986; Hoar, 1988). Dickhoff *et al.* (1982) found that both yearling and underyearling (zero-age) coho salmon in freshwater showed spring and/or summer increases in plasma thyroid hormone concentrations which correlated with the fish smoltifying. Successful adaptation to sea water of zero-age coho coincided with the progressive rise in plasma tri-iodothyronine and for the yearlings with the progressive rise of plasma thyroxine. They also observed a similar trend in thyroid hormone peaks and sea water survival in quinnat salmon.

Using freshwater variables indicating the fish's physiological state with respect to hypoosmotic regulatory ability is effective provided that the transfer and seawater conditions are 'ideal'. Just monitoring the freshwater salmon does not take into account sea water conditions such as temperature which has a considerable effect on the salmon's ability to successfully osmoregulate in sea water (see Chapter 6). However, measuring changes in salmon transferred to seawater takes into consideration both the physiological state of the salmon as well as transfer and sea water conditions, so giving a more conclusive and

accurate account of the salmon's hypoosmotic regulatory ability. This is the basis of the seawater challenge test described and vindicated by Clarke and Blackburn (1977).

The best indicator of sea water survival in this study was plasma chloride or sodium levels in salmon sampled after 24 hours in sea water. The differences between the chloride concentrations of successful compared with unsuccessful sea water transfers were large and more easily discernible than the freshwater chloride samples of similar transfers. Differences in the changes in plasma cortisol concentrations of 24 hour sampled salmon were greater than the freshwater changes and could also be used as an effective indicator of the osmoregulatory status of the salmon. Finally it should be noted that changes in the timing of smoltification and in seawater survival differed between brood years and seasons and so predictions made for one year can not be assumed to be the same for the following year.

CHAPTER 6

Effect of Seawater Temperatures on the Survival of Seawater Transferred Sockeye and Quinnat Salmon.

INTRODUCTION

Fish have been found to occur in water temperatures that range from -2.0°C to 44°C, although no one species of fish has been found to survive this full range of temperatures (Elliott, 1982). Individual species of fish can only live within certain limits and outside these the fish would be physiologically stressed or killed. The thermal lethal limits of fish indicate the maximum extremes that the fish can tolerate, but these limits do not necessarily indicate the range of temperatures best suited for growth and reproduction. Salmonids have a relatively small range of temperatures in which they can survive, especially when compared to the goldfish, *Carassius auratus*, which is able to withstand temperatures ranging from close to freezing point up to 41° C (Elliott, 1981). Brett (1952) found that the upper lethal temperature for juvenile quinnat salmon (*Oncorhynchus tshawytscha*) was 25.1°C and for juvenile sockeye salmon (*Oncorhynchus nerka*) it was slightly lower, at 24.4° C. However, these temperature limits are only valid if there are no other variables (stressors) affecting or disrupting the fish's homeostasis. The thermal tolerance of a fish is reduced if it is, at the same time, being influenced by other stressors (Wedemeyer and McLeay, 1981).

A critical phase during the life history of an anadromous salmonid is the transition from fresh water to sea water. In sea-cage rearing operations, the transfer of salmon from fresh water to sea water is often abrupt and high mortalities can result if the timing of the transfer is not right. In such aquacultural practices, the abrupt seawater transfer would act as a stressor. To survive the large increase in salinity, the salmon changes from being a hyperosmotic regulator to a hypoosmotic regulator, a complete reversal of the fish's osmoregulatory mechanisms. Prior to migrating downstream to the ocean, juvenile salmon undergo preparatory modifications which permit them to successfully adapt to the marine environment (Hoar, 1976; Langdon and Thorpe, 1984; Hoar, 1988). This is known as the parr-smolt transformation or smoltification. Successful adaptation to the new medium is dependent on a variety of factors, including the physiological state of the salmon prior to

transfer (the degree of smoltification) and environmental conditions (Wedemeyer *et al.*, 1980). This study investigates the effect of one environmental factor, seawater temperature, on the direct transfer of sockeye and quinnat salmon to sea water. The salmon were transferred to 13°C and 19°C sea water and survival and physiological changes monitored. This range of sea water temperatures can be encountered along the New Zealand coastline where sea-cage rearing operations are situated. Although other studies have investigated the effect of water temperature (both fresh water and sea water) on the seawater adaptability of salmonids, most of these studies have looked at upper temperatures considerably lower than 19°C (Knutsson and Grav, 1976; Clarke *et al.*, 1978; Clarke *et al.*, 1981; Clarke and Shelbourn, 1985; Pereira and Adelman, 1985; Johnsson and Clarke, 1988).

Material and Methods

Fish Stock

Sockeye salmon were obtained from a pond rearing farm at Prebbleton, 14km south of Christchurch, New Zealand. Quinnat salmon were obtained from the Prebbleton farm and from a Ministry of Agriculture and Fisheries hatchery situated on the Glenariffe stream, a tributary of the Rakaia river. Salmon were transported to the Edward Percival Field Station, Kaikoura where seawater transfer experiments were performed. Fish were housed in 80 litre aquaria at a density no greater than 6-10 g fish per litre and maintained under a natural photoperiod. All experiments were performed at least 4 days after the transportation of salmon to Kaikoura.

Effect of Seawater Temperatures on Seawater Survival of Salmon

Physiological data and survival were monitored in salmon that were transferred into sea water at two set temperatures: 13°C and 19°C. Transfers were made in February 1987, when the ambient sea water temperature was 19°C, and in June 1987, when the temperature was 13°C. In February, salmon were also transferred into sea water that had been cooled to 13°C, and in June, salmon were transferred to sea water heated to 19°C. Two size cohorts of quinnat were tested in February (mean weights of 8.8±2.4g and 18.5±4.0g). Seawater survival of both sockeye and quinnat salmon was investigated, although physiological measurements were only made from the larger cohort of (18.5g) quinnat salmon. In February 1987, two size classes of quinnat were studied. Table 1 summarises the different seawater transfers performed. All salmon were abruptly

Table 1. Experimental temperature protocols and the mean weights of fish directly transferred from fresh water into sea water.

	Fish Weight (g) (x ± S.D.)	Transfer Regime F.W. Temp.	S.W. Temp.
February 1987			
Sockeye	21.2±1.6	13°C 13°C	13°C 19°C
Quinnat	8.8±2.4	13°C 13°C	13°C 19°C
Quinnat	18.5±4.0	13°C 13°C	13°C 19°C
June 1987			
Sockeye	84.3±8.9	13°C 13°C	13°C 19°C
Quinnat	67.2±14.5	13°C 13°C	13°C 19°C

transferred to sea water from 13°C fresh water. Fresh water was replaced with sea water within 20 minutes and all transfers were started between 09 00 and 10 00 hours.

Controls

For controls, salmon were transferred from 13°C fresh water to 19°C fresh water. Only survival was monitored, no physiological measurements being taken.

Sampling and Sample Analysis

Salmon were sampled initially in fresh water, then at 1.5, 4, 12, 24, 48, 96 hours and 12 and 30 days after sea water transfer. To sample, fish were randomly netted from the aquaria and rapidly anaesthetised within 30 seconds in 2-phenoxyethanol (4-6mls per litre 30% seawater). The fish were removed from the anaesthetic, excess fluid blotted off, and the salmon weighed and measured for determination of Fulton's Condition Factor (C.F.= $100W/l^3$, where W= weight in grams and l= length in centimetres). The caudal fin was severed and blood collected from the caudal vasculature into heparinised (ammonium heparinate) hypodermic syringes. No more than seven fish were sampled at one time and such a sample would take less than 10 minutes. Once all the fish had been sampled, the blood was transferred from the syringes to Eppendorf centrifuge tubes. A small aliquot of whole blood was withdrawn into a micropipette, sealed at one end, and centrifuged at 20 000g for 3 minutes to determine haematocrit. The blood remaining in the Eppendorf tube was spun at 5000g for 3 minutes, and the plasma removed and stored in plastic vials at -80°C until analysed.

A sample of white myotomal muscle was taken from the dorso-lateral region, just

below and behind the dorsal fin. A sharp scalpel was used to separate the skin plus any red muscle from the white myotome. Dissected muscle samples were immediately placed onto a preweighed square of tin foil and weighed. The tin foil was wrapped around the muscle and placed into a 70°C oven until a constant dried weight was attained (approx. 48 hours). The percentage water content was calculated.

Gill tissue was excised from the salmon, washed in homogenising buffer (see Chapter 2) and stored in plastic vials at -80°C. This tissue was analysed for Na^+-K^+-ATPase activity by a modification of the methods used by Johnston *et al.* (1977) and Langdon *et al.* (1984) (see Chapter 2 for details).

Plasma samples were analysed for chloride concentrations and osmolarity. Chloride concentrations were determined from duplicate or triplicate 10µl plasma samples that were analysed with a CMT 10 Radiometer chloride titrator. Osmolarity was measured with a Wescor 5100C vapour pressure osmometer using duplicate 8µl plasma samples.

Data Analysis

Results are expressed as the mean standard error. Statistical significance was tested using either the Chi Square test for heterogeneity, the Student's t-test, or analysis of variance in conjunction with Duncan's multiple range test. Data that were not normally distributed were transformed before statistical treatment.

Results

Seawater Survival of Salmon

Sockeye that were transferred into sea water in February 1987 (mean weight = 21.2 ±1.6g) failed to adapt in either the ambient (19°C), or the cooled sea water (13°C) (Fig. 1a). Nevertheless, sockeye that were transferred into the cooled sea water survived 24 hours longer than the sockeye transferred into the sea water at ambient temperature. One hundred percent mortality occurred after 4 days in the sockeye transferred to the cooler sea water. In June 1987, when the sockeye of a much larger size (mean weight = 84.3±8.9g) were transferred into sea water there was a large increase in survival (Fig. 1b). There was no mortality in the sockeye transferred into the ambient sea water (13°C) and 30% mortality occurred in the sockeye transferred into the heated sea water (19°C)

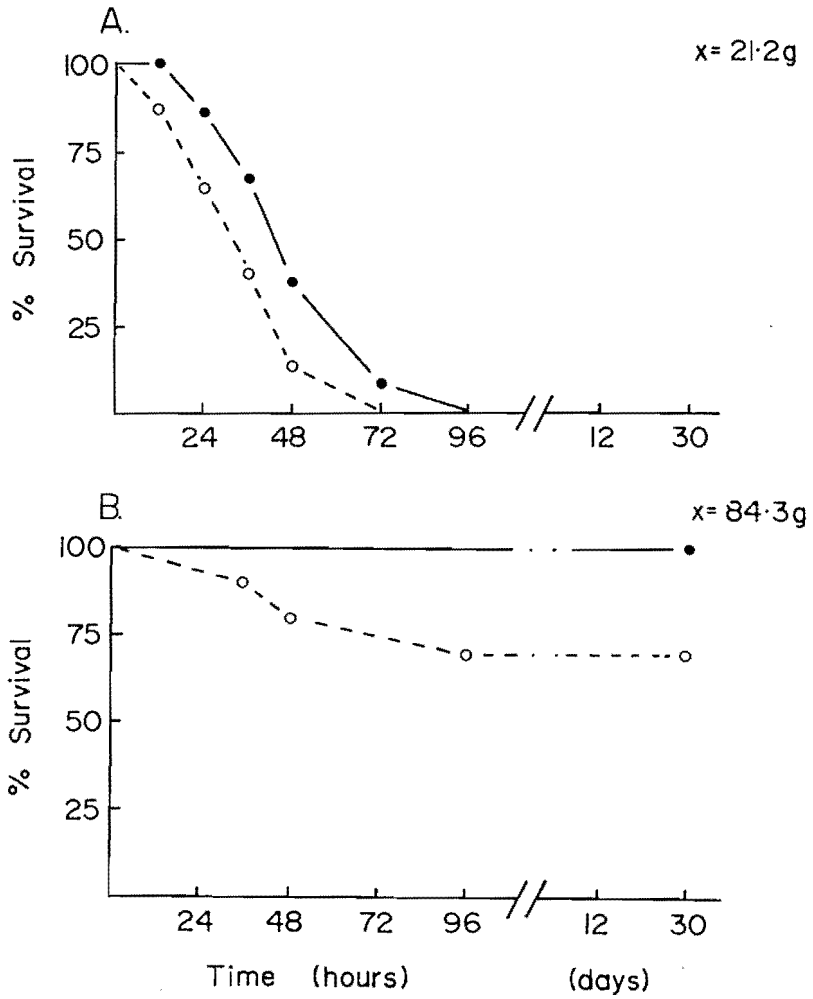

Figure 1. Survival curves for sockeye salmon transferred into 13 C (●) and 19 C (O) sea water in (A) February 1987 and (B) June 1987.

which were significantly different ($p<0.05$). Salmon in both the June transfers had grown after 30 days in sea water.

The gill Na^+-K^+-ATPase activities of the sockeye before transfer in February and June were 1.50±0.29 and 3.70±0.57 μmoles phosphate mg protein^{-1} hour^{-1}, respectively, the June sockeye gill ATPase activity being significantly greater ($p<0.01$).

Two sizes of quinnat were transferred into ambient (19°C) and cooled sea water (13°C) in February 1987 (Fig. 2a&b). The smaller quinnat (mean weight = 8.8±2.4g) suffered higher mortality than the larger quinnat (mean weight = 18.5±4.0g), and for both size classes higher mortality occurred in the salmon transferred to the 19°C sea water (Fig. 2a & b). All of the smaller quinnat were dead after 3 days in 19°C sea water while there was 83.3% mortality after 30 days in the larger quinnat. Of the smaller quinnat transferred into the cooled sea water,

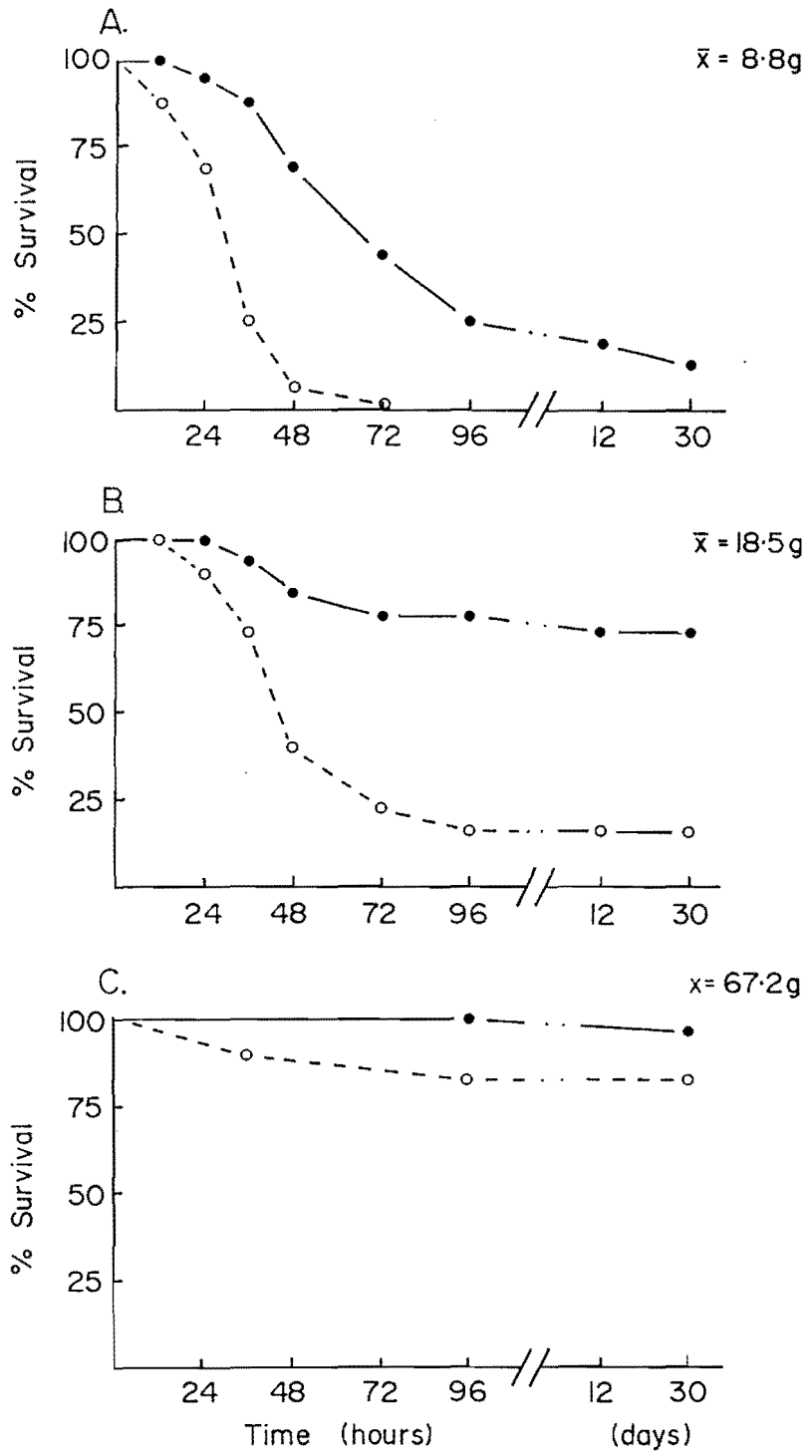

Figure 2. Survival curves for quinnat salmon transferred into 13 C (●) and 19 C (O) sea water in (A & B) February 1987 and (C) June 1987.

only 12.5% were alive after 30 days, compared with 72.2% survival for the larger quinnat. In the larger size group of quinnat, those that survived the seawater transfers in February 1987 had a body weight greater than 15.0g. Conversely, the fish that died were under 12.0g. With regard to the smaller size class of quinnat, the 12.5% that survived in sea water for 30 days had a final mean weight of 16.9± 4.3g. There was a direct relationship between fish weight and the time of death after transfer to sea water. Growth only occurred in the larger cohort of quinnat transferred to the cooler 13°C sea water.

In June 1987, only one size class of quinnat (mean weight = 67.2±14.5g) was transferred into ambient (13 °C) and heated (19 °C) sea water (Fig. 2c), with 95% and 85% survival, respectively. There was no significant difference in the survivorship of the quinnat transferred to the two seawater temperatures (Chi Square = 1.111). Both groups of fish had grown after 30 days in sea water.

In February, the smaller quinnat had a gill Na^+-K^+-ATPase activity of 2.11±0.54 and the larger quinnat an activity of 2.97±0.59 umols phosphate mg protein^{-1} hour^{-1}, which were not significantly different from each other. The gill Na^+-K^+-ATPase activity of the quinnat transferred in June (4.51± 0.70 umols phosphate mg protein^{-1} hour^{-1}) was significantly greater than the February-transferred quinnat ($p<0.05$).

Physiological Changes in Quinnat Transferred to Seawater

<u>February 1987 Seawater Transfers</u>

In February 1987, plasma chloride concentrations increased in quinnat transferred to 13°C and 19 °C sea water (Fig. 3). There was a greater increase in chloride levels in the quinnat transferred to the 19° C sea water, with a peak of 192±7 mmol l^{-1} chloride, recorded 24 hours post-transfer. This was greater than the maximum chloride concentration of 171±7 mmol l^{-1}, recorded 24 hours after quinnat were transferred into 13 °C sea water ($p<0.01$). The chloride concentrations for the two transfers remained significantly different until 96 hours post-transfer ($p<0.05$). After 30 days, the concentrations were the same for the two seawater transfers and were significantly greater than the pre-transfer levels (both, $p<0.01$) (Fig. 3).

The changes recorded in osmolarity were similar to the changes in chloride concentrations. Osmolarity increased in quinnat that were transferred to the 13 °C and the 19 °C seawater and a maximum osmolarity was recorded for both transfers at 24 hours (Fig. 3). There was a greater increase in osmolarity in

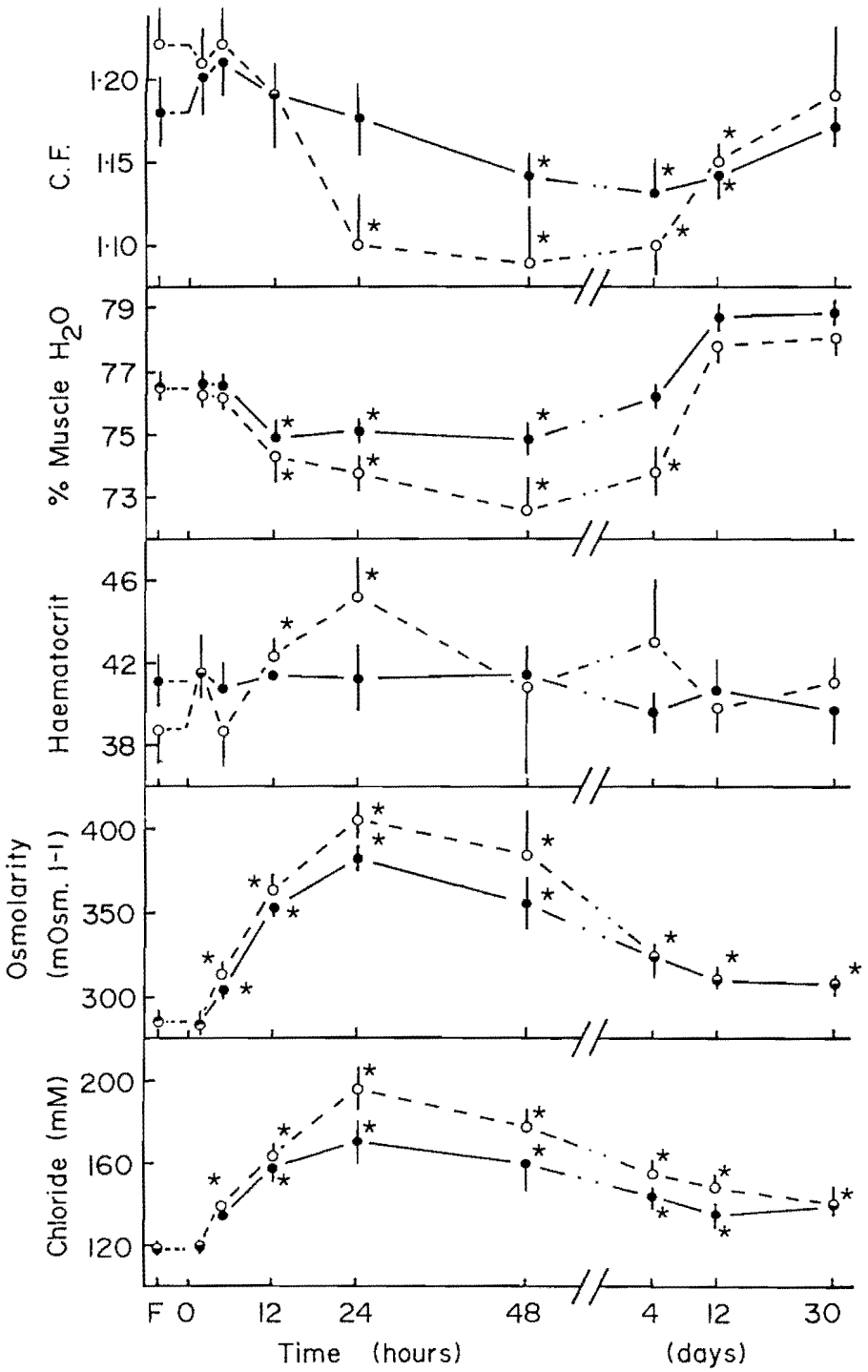

Figure 3. Physiological changes in quinnat salmon transferred to 13 C (●) and 19 C (○) sea water in February 1987. F - initial freshwater sample. All results are means±S.E. Asterisks indicate a significant difference from initial freshwater samples (Duncan's multiple range test, $P<0.05$).

the quinnat transferred to the 19°C sea water at 24 hours post-transfer ($p<0.05$). After 24 hours the osmolarity decreased and then stabilised at levels that were significantly higher than the pre-transfer values ($p<0.01$, Fig. 3).

There was no significant change in the haematocrit of quinnat transferred to the 13°C sea water (Fig. 3). However, the haematocrit of quinnat transferred to 19°C sea water, significantly increased from a pre-transfer value of 38.8±1.6% to 45.2±1.8%, 24 hours after seawater transfer ($p<0.01$) (Fig. 3). The haematocrit then returned to basal levels within 30 days post-transfer.

The percentage water content of the myotome (white muscle only) significantly decreased after 12 hours in quinnat that were transferred into both the 13°C and 19°C sea water ($p<0.01$) (Fig. 3). In the quinnat that were transferred to the 13°C sea water, the percentage water content remained at about 75% until 48 hours post-transfer and then increased to a maximum value of 78.5±0.2% 30 days post-transfer (Fig. 3). This was significantly greater than the pre-transfer value ($p<0.01$). The percentage muscle water content of quinnat transferred to 19°C sea water decreased to a recorded minimum of 72.7±1.0% 48 hours after transfer, and this was significantly lower than the percentage muscle water content of quinnat that had been transferred to the 13°C sea water. After 48 hours, the percentage muscle water content increased in quinnat transferred to the 19°C and stabilised at levels that were similar to the 13°C seawater transferred quinnat at 30 days (Fig. 3).

The condition factor of the quinnat transferred to the sea water at 13°C slowly decreased and was significantly lower than pre-transfer values after 48 hours ($p<0.05$). By 30 days, the condition factor had returned to pre-transfer levels. In the quinnat transferred to the 19°C sea water, the condition factor decreased more markedly from 1.22±0.02 to a minimum of 1.09±0.03 at 48 hours, the latter being significantly lower than the condition factor of the 13°C seawater transferred quinnat sampled at the same time. The condition factor remained at this level until 4 days, after which it increased and returned to pre-transfer values by 30 days (Fig. 3).

June 1987 Seawater Transfers

The changes in quinnat after transfer to 13°C and to 19°C sea water in June 1987 were generally smaller in magnitude than the changes recorded for the quinnat transferred to sea water in February 1987. This was especially so in the quinnat introduced into the 19°C sea water. Moreover, the differences between

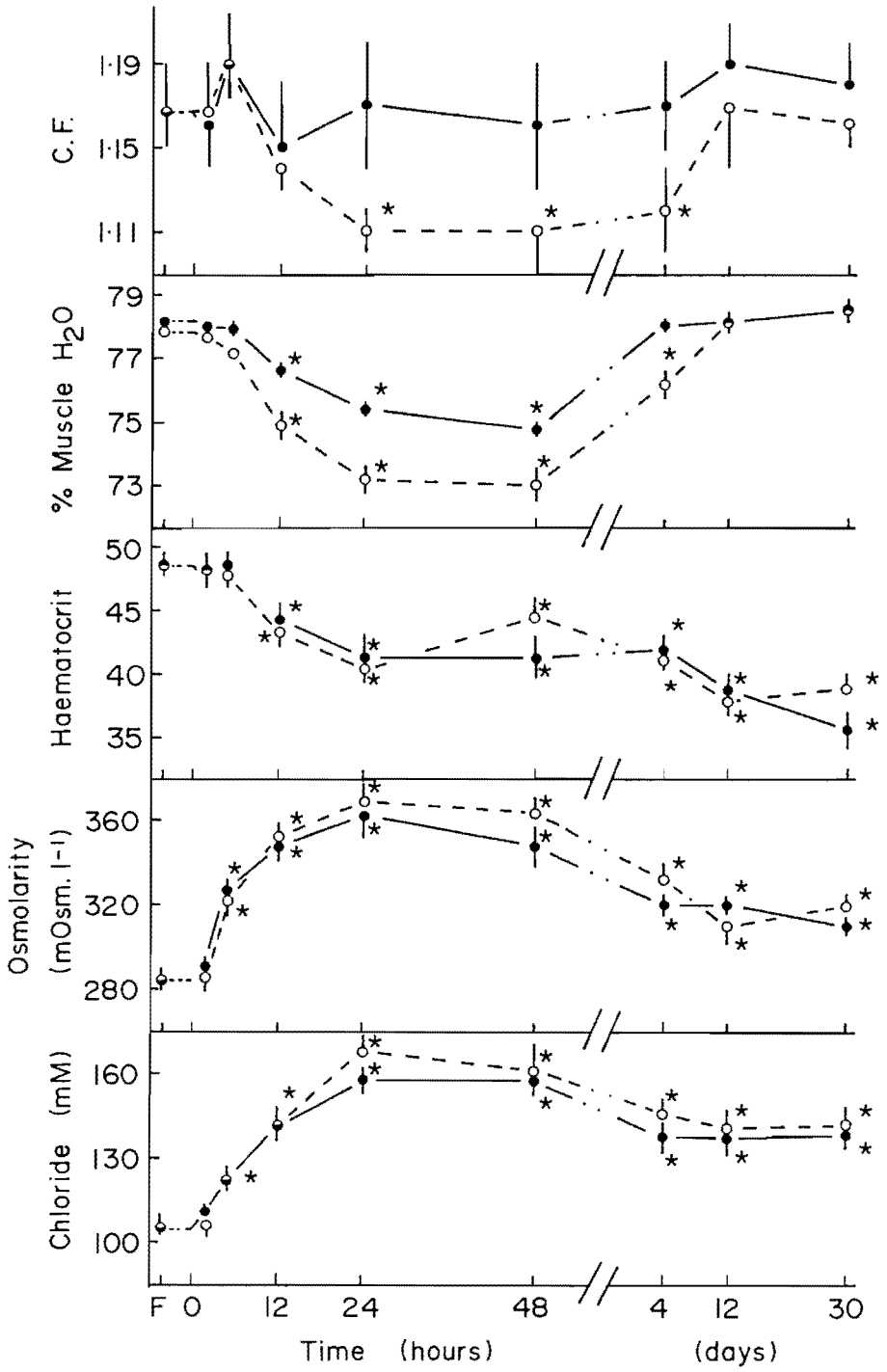

Figure 4. Physiological changes in quinnat salmon transferred to 13 C (●) and 19 C (O) sea water in June 1987. F - initial freshwater sample. All results are means±S.E. Asterisks indicate a significant difference from initial freshwater samples (Duncan's multiple range test, $P<0.05$).

the 13°C and 19°C seawater transferred salmon at this time were not so marked compared with the differences between these temperatures in February. For comparative purposes, it should be noted that some of the y axis scales are different on Fig. 4 compared with Fig. 3.

Plasma chloride concentrations rose significantly after quinnat were transferred to 13°C and 19°C sea water ($p<0.01$), and maxima of 158±3 mmol l^{-1} (13°C) and 166.5±5 mmol l^{-1} (19°C) were recorded after 24 hours (Fig. 4). There was no significant difference between the chloride concentrations of quinnat transferred to the 13°C or 19°C sea water. Compared with the changes in chloride levels recorded in quinnat transferred to 19°C sea water in February, the maximum level in June was considerably lower (Figs. 3 and 4). In February, a maximum chloride concentration of 192±7 mmol l^{-1} was recorded 24 hours post transfer in the 19°C seawater transferred quinnat, and this was significantly greater than the maximum of 166±5 mmol l^{-1} chloride recorded in June ($p<0.01$).

Similarly to the changes in chloride concentrations, the plasma osmolarity of quinnat transferred into 13°C and 19°C sea water increased, and for both transfers a peak osmolarity was recorded after 24 hours (Fig. 4). This was followed by a decrease that stabilised to approximately 320 mosmol l^{-1} 4-12 days post-transfer. There was no significant difference between the changes in osmolarity in quinnat transferred to 13°C or to 19°C sea water. The changes in osmolarity in the 19°C transferred salmon were greater in February than in June (Figs. 3 and 4).

Following the June transfers haematocrit steadily decreased to reach 35.6±1.5% (13°C) and 38.2±1.2 (19°C) after 30 days (Fig. 4). There was no significant difference in any of the changes in haematocrit between the two temperatures.

In quinnat transferred to 13°C and 19°C sea water, there was a significant decrease in the % water content of the myotome at 12 hours ($p<0.01$, Fig. 4). This was followed by a return to pre-transfer values after 4 (13°C) and 12 days (19°C). A significantly greater decrease occurred in the quinnat transferred to the 19°C sea water (Fig. 4).

The condition factor of quinnat transferred into 13°C sea water did not significantly change throughout the duration of the experiment. In contrast, the condition factor of quinnat transferred into 19°C sea water significantly decreased and remained lower than pre-transfer values until 12 days

post-transfer. The condition factor was significantly lower in the 19°C sea water transferred quinnat than in the quinnat transferred to 13°C.

No mortalities occurred in the control salmon that were transferred from fresh water to fresh water (either the 13°C to 13°C or 13°C to 19°C transfers).

Discussion

The ability of quinnat and sockeye salmon to survive and successfully osmoregulate in sea water was related to high sea water temperatures. High temperatures led to high mortalities, although it did not completely prohibit a successful sea water transfer. Quinnat and sockeye salmon were able to successfully adapt to, and grow in, 19°C sea water provided that they were large enough and that they had undergone sufficient preparatory modifications for ocean residence while in fresh water.

Clarke and Shelbourne (1985) investigated the effect of both fresh water and sea water temperatures on the seawater adaptability of juvenile, fall quinnat salmon (Nth American). They studied a large number of different temperatures, but only considered sea water temperatures up to 14.5°C. They found that the optimum regulation of plasma sodium occurred with the transfer of 5.6g quinnat from 13.8°C fresh water to 10.2°C sea water. Both freshwater and seawater temperatures in New Zealand are generally much higher than those in Canada, thus any work carried out in New Zealand should be above these Northern Hemisphere optima. The present study was not designed to determine optimal growing temperatures, but rather to determine the ability of the fish to survive transfer to very warm (19°C) sea water, as is commonly found in New Zealand.

Differences occurred between the ability of the sockeye and quinnat to adapt to sea water. Sockeye were generally not as successful in surviving the transition to the 19°C sea water. However, the gill Na^+-K^+-ATPase activities of sockeye were lower than in quinnat for all transfers so the decreased survival may be due to this factor. If the sockeye had been transferred to sea water with gill Na^+-K^+-ATPases activities as high as quinnat then the same survival trends could have resulted for both species. Nevertheless, it should be noted that Brett (1952) found that the lethal upper temperature limit of sockeye (24.4°C) was lower than quinnat (25.1°C), which suggests that sockeye could be

more liable to fail a transfer at high temperatures.

An increase in gill Na^+-K^+-ATPase activity has been used as an indicator of smoltification in salmonids (Hoar, 1988; see Chapter 5). It is during smoltification that the juvenile salmon undergo preparatory modifications which enable them to adapt successfully to sea water. Gill Na^+-K^+-ATPase activity levels are in a continual state of flux. The level of enzyme activity needed for successful seawater adaptation appears to be dependant on the other factors affecting the salmon during transfer. Gill Na^+-K^+-ATPase activity does not have to peak for the salmon to successfully adapt to sea water as was shown when quinnat (the larger cohort, average weight = 18.5g) successfully transferred to the 13°C sea water in February 1987. These salmon had considerably lower enzyme activities than the quinnat transferred in June 1987, yet both were successful in adapting to the 13°C sea water. However, when the same species were transferred to the 19°C sea water in June, only the quinnat with the higher enzyme activities were able to make the transition successfully. The salmon with the higher enzyme activities are better prepared for the seawater transfer and appear to be able to compensate for the higher sea water temperatures.

The changes in plasma chloride concentrations, osmolarity, muscle water content and condition factor of quinnat transferred to sea water lend support to the role of elevated gill Na^+-K^+-ATPase activities as a prerequisite for the successful transfer of salmon to sea water. Smaller deviations away from the initial freshwater values occurred in the June seawater-transferred quinnat (which had higher ATPase activities) than the February transferred quinnat. For both groups transfer to the 19°C sea water caused a larger osmotic imbalance than the quinnat transferred to the 13°C sea water. The fish suffered greater tissue dehydration and elevation of plasma chloride concentration and osmolarity.

Not only do high temperatures affect survival but low seawater temperatures can also affect the seawater adaptability of salmonids. Finstad *et al.* (1988) found that rainbow trout (*Salmo gairdneri*) transferred to 1°C sea water suffered a reduction in the capacity to osmoregulate and all died with 7 days of transfer.

The greater osmotic imbalance associated with high sea water temperatures could be due to the physical properties of high temperatures and/or a result

of the high temperatures acting as an additional stressor to the seawater transferred salmon. High temperatures typically increase diffusion rates and can also increase the permeability of membranes by affecting the lipid bi-layer (Prosser, 1973) Both of these would increase the influx of ions into the fish upon sea water transfer. Elevated temperatures may also have an effect on the functioning of the gill Na^+-K^+-ATPase enzyme which increases in activity on sea water transfer (see Chapter 4 for details). High fresh water temperatures (above 13°C) have been shown to inhibit or reduce the elevation in this enzyme during smoltification (Zaugg, 1981; Hoar, 1988), so it is conceivable that high sea water temperatures may also inhibit or slow down the increase of this enzyme after seawater transfer.

An increase in temperature has been shown to elicit a stress response in salmon (see Chapter 3) and an osmotic imbalance often results. Assuming that the high sea water temperature is acting as an additional stressor it would further increase the osmotic imbalance associated with the transfer of salmon to sea water. It should be noted that mortality on transfer could be the result of the temperature change rather than the 19°C per se.

In conclusion, high sea water temperatures (19°C) can reduce the ability of quinnat and sockeye salmon to successfully adapt to sea water. However, as long as the salmon are adequately prepared for the transition into sea water (fully smoltified) they are capable of adapting to sea water temperatures as high as 19°C.

CHAPTER 7

Comparison of Direct and Progressive Seawater Transfers in Quinnat and Sockeye Salmon.

Introduction

Salmon migrating downstream to the ocean would generally have the opportunity to stay for a period in the boundary zone which encompasses salinities intermediate to fresh water and sea water. However, in commercial salmon farming operations such as sea-cage rearing, the salmon are placed directly from fresh water into sea water. The osmotic shock imposed by an abrupt transfer to sea water would be considerably greater than if the salmon encountered a gradual change in salinity, and this could have an influence on the ability of the salmon to adapt successfully to the hypertonic medium. The abrupt transfer to sea water would act as a much larger stressor than a progressive transfer. After direct sea water transfer there is an initial increase in the concentration of plasma ions and osmolarity (see Chapter 4 for details). If this deviation away from regulated levels is too great, the result is often severe dehydration and death. The extent of this osmotic imbalance is in part due to the external salinity; therefore, if the salmon is exposed to a slow increase in salinity the osmotic disturbance could be reduced, enabling the salmon more time to activate hypoosmoregulatory mechanisms.

This study compares the survival of quinnat (*Oncorhynchus tshawytscha*) and sockeye (*O. nerka*) salmon following direct and progressive transfer into sea water. Physiological changes in sockeye salmon after seawater transfer were monitored and the two types of transfer compared.

Materials and Methods

Fish Stock

Sockeye salmon and quinnat salmon were obtained from a Ministry of Agriculture and Fisheries hatchery situated on the Glenariffe stream, a tributary of the Rakaia river. Seawater transfer experiments were performed at the Edward

Percival Field Station, Kaikoura. Fish were housed in 80 litre aquaria at a density no greater than 6-10 g fish per litre and maintained under a natural photoperiod. All experiments were performed at least 4 days after the transportation of salmon to Kaikoura.

Experimental Design

Effect of Rate of Seawater Transfer

Two types of transfer were made. Salmon were either abruptly transferred from fresh water to sea water (direct transfer), or they were exposed to a gradual increase in salinity (progressive transfer). Both sockeye and quinnat salmon were transferred into sea water by these two methods in September, October and November 1985. The mean weights of the salmon and the water temperatures are shown in Table 1.

Table 1. Water temperatures and the age and mean weights of fish transferred from fresh water into sea water.

Transfer Date	Species	Fish Weight (x ± S.D.)	Water Temps.(°C) F.W.	S.W.
September 1985	Sockeye	1.30±0.27	11.0	11.8
	Quinnat	0.23±0.02		
October 1985	Sockeye	3.54±0.67	12.0	12.7
	Quinnat	0.44±0.22		
November 1985	Sockeye	6.84±0.70	13.8	13.6
	Quinnat	1.29±0.67		

The direct transfer of salmon was achieved by displacing fresh water with sea water flowing in at a rate of 10 –12 litres per minute. Fresh water was totally exchanged with sea water (salinity = 34‰) within 20 minutes, after which the flow was reduced to 2-4 litres per minute. The progressive transfer of salmon into 100% sea water was completed within five days and was conducted in well aerated, static water. The salinity was increased in discrete jumps (10‰ , 17 ‰, 25‰ , 30 ‰, and 34‰) at 24 hourly intervals. Once a salinity of 34 ‰ was achieved, sea water was continually exchanged with new sea water (flow rate = 2-4 litres per min.). All sea water transfers were started between 0900 and 10 00 hours. Tanks were checked at 12 hourly intervals for mortalities and the number of dead fish and their respective weights noted.

Physiological data was collected from sockeye salmon transferred into sea water in November 1985. For the direct transfer, the salmon were sampled in fresh water, and at 1, 2, 4, 12, 24, 48, 96 hours and 12 days post transfer. The

salmon that were progressively transferred to sea water were sampled in fresh water, and after 2 and 24 hours for each salinity. Once into 100 percent sea water, the salmon were sampled at 48 hours and after 6 days.

Sampling and Sample Analysis

To sample, fish were randomly netted from the aquaria and rapidly anaesthetised within 30 seconds in 2-phenoxyethanol (4-6mls per litre 30% seawater). The fish were removed from the anaesthetic, the excess water blotted off, and the salmon weighed and measured for determination of Fulton's Condition Factor (C.F.= $100W/l^3$, where W= weight in grams and l= length in centimetres). The caudal fin was severed and blood was collected from the caudal vasculature with heparinised (ammonium heparinate) hypodermic syringes. No more than seven fish were sampled at one time and the total sample would take less than 8 minutes. Once all the fish had been sampled, the blood was transferred from the syringes to Eppendorf centrifuge tubes and spun at 5000g for 3 minutes. The plasma was removed and stored at -80°C in plastic vials until analysed.

Plasma chloride concentrations were determined from duplicate or triplicate 10µl plasma samples that were analysed with a Radiometer chloride titrator. Plasma cortisol concentrations were measured by radioimmunoassay (see Chapter 2 for details).

Data Analysis

Results are expressed as the mean standard error. Statistical significance was tested using either the Chi Square test for heterogeneity, the Student's t-test, or analysis of variance in conjunction with Duncan's multiple range test. Data that were not normally distributed were transformed before statistical analysis.

Results

Comparison of Direct with Progressive Seawater Transfer

Quinnat salmon

No quinnat survived either the direct or progressive sea water transfer in September and October 1985 (Fig. 1). In November 1985, only 10.3% of the quinnat were alive 30 days after direct transfer to seawater, and for the progressively transferred quinnat, 25% survived. The two treatments were not, however, significantly different from each other.

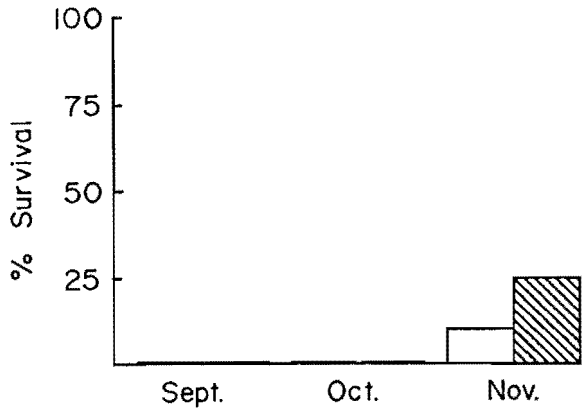

Figure 1. Survival after 30 days in quinnat salmon transferred directly (plain histogram) and progressively (hatched) into sea water.

The rate of mortality for the different transfers are shown in Figs. 2a-f. In September, quinnat transferred directly into sea water were all dead by 24 hours (Fig. 2a). The majority of the quinnat transferred progressively into sea water in September died while still in the intermediate salinities and 100% mortality occurred by 12 hours in 100% sea water (Fig. 2b). Quinnat transferred directly or progressively into sea water in October showed similar trends to the September transferred quinnat, although the October fish survived slightly longer in sea water (Fig. 2c & d).

In November, there was a more gradual decrease in survival of quinnat transferred directly into sea water (Fig. 2e). Four days post-transfer, 34% of the salmon were still alive, though this had decreased to 10.3% by 21 days. No growth occurred in these remaining salmon and some appeared moribund. In the progressive transfer, the majority of the mortalities occurred after the quinnat had reached 100% sea water and once the salmon were in this salinity, the mortality rate was more rapid compared to the direct transfer of quinnat (Fig. 2f). There was also no growth in these remaining salmon.

Sockeye salmon

There was no difference in the survivorship of sockeye transferred directly or progressively into sea water in September 1985 and November 1985 (Fig. 3) In September, the transferred sockeye suffered high mortalities for both types of transfer, whereas in November the opposite occurred, and there was low mortality in both transfer groups (Fig. 3). In October, there was a significant difference between the direct and progressive transfer of sockeye salmon, with respect to seawater survival ($p<0.05$) (Fig. 3). There was lower mortality in the sockeye that were progressively transferred into seawater.

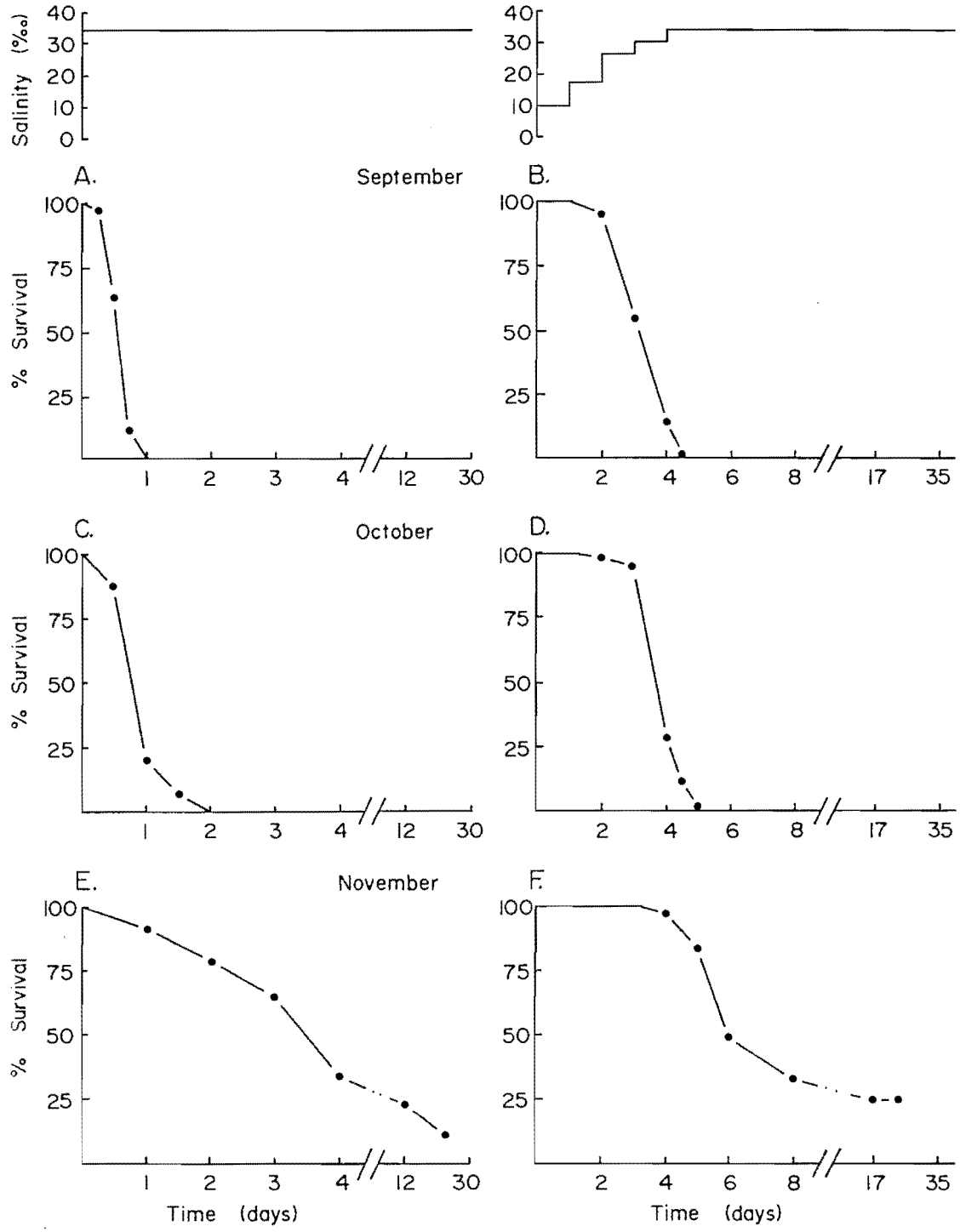

Figure 2. Survival curves for quinnat salmon transferred into sea water directly (left column, A, B & C) and progressively (right column B, D & F). Quinnat were transferred in September 1985 (A & B), October 1985 (C & D) and November 1985 (E & F).

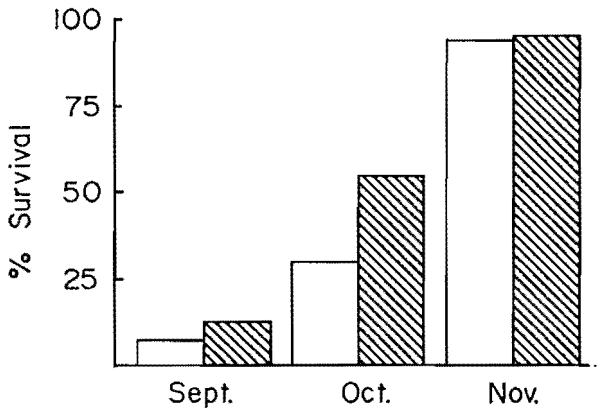

Figure 3. Survival after 30 days in sockeye salmon transferred directly (plain histogram) and progressively (hatched) into sea water.

The rate of mortality in sockeye transferred in September was the most rapid compared with the other months (Fig. 4a-f). In September, four days after direct seawater transfer 93% of the sockeye were dead, although the fish that remained at this time were still alive after 30 days (Fig. 4a). No mortality occurred in the progressively transferred sockeye until the fish were placed into a salinity of 34‰ , after which the mortality rate was faster than the direct transfer (Fig. 4b). Neither the direct or the progressively transferred salmon increased in size while resident in sea water.

Direct transfers of sockeye into fresh water during October caused a steady and continual loss of fish throughout the course of the experiment (Fig. 4c) and some that remained after 30 days appeared moribund. The progressively transferred sockeye, initially showed a similar rate of mortality to the directly transferred salmon once the salmon were in a salinity greater than 30‰ (Fig. 4d). In contrast to the direct transfer, there was no mortality 17 days post-transfer, although some sockeye appeared moribund. No growth occurred in the direct or progressively transferred sockeye.

In November, similar survivorship occurred for the sockeye transferred into sea water both progressively and directly (Fig. 4e & f).

Direct transfer of sockeye to sea water produced a rapid and significant increase in plasma cortisol concentration ($p<0.01$) (Fig. 5). A peak of 169±22 ng ml^{-1} cortisol was recorded 1 hour post-transfer and this was followed by a decrease to basal levels by 48 hours post-transfer. Plasma chloride levels significantly increased after sockeye were transferred directly into sea water ($p<0.01$) (Fig. 5). Twenty four hours after seawater transfer, a maximum chloride

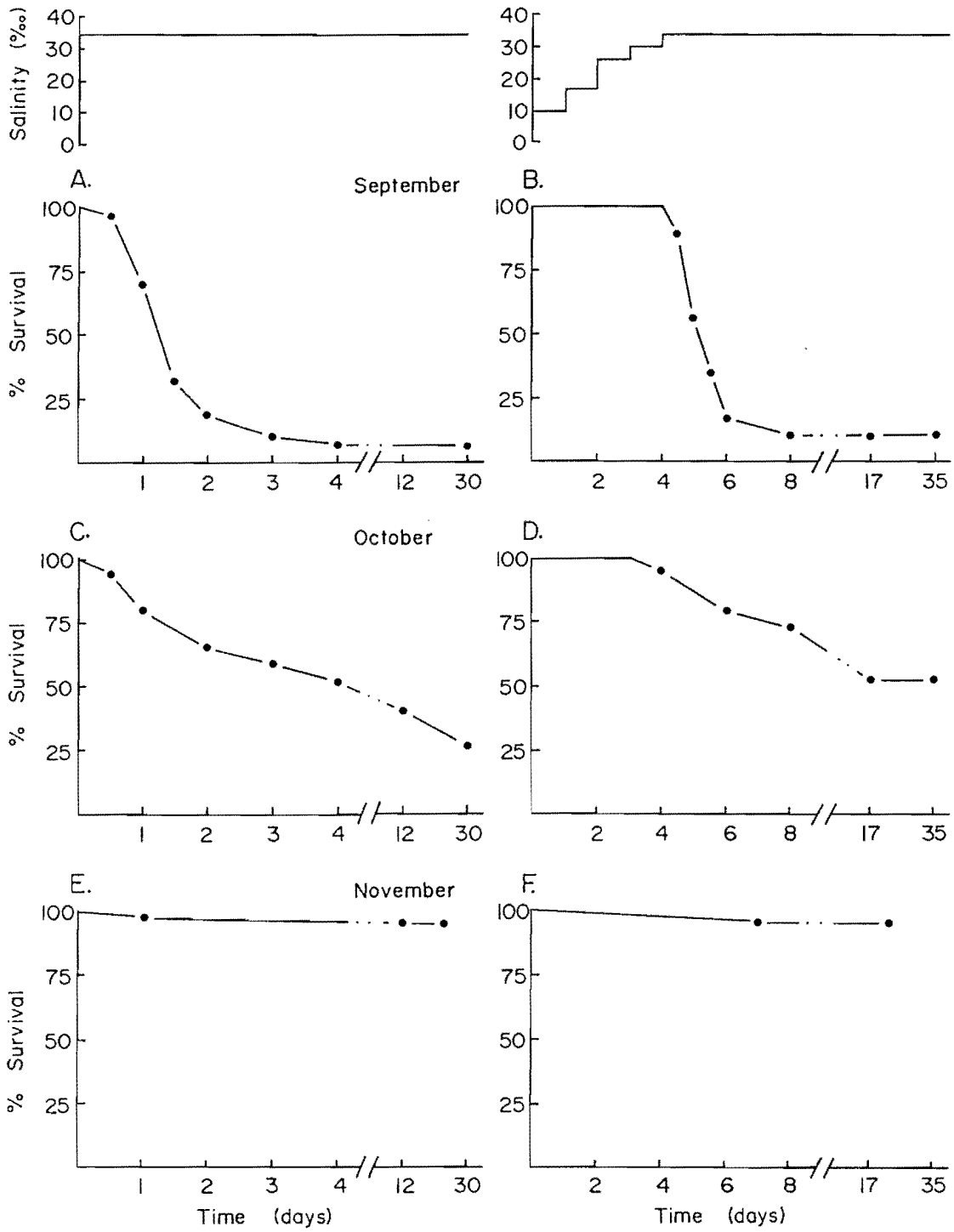

Figure 4. Survival curves for sockeye salmon transferred into sea water directly (left column, A, B & C) and progressively (right column B, D & F). Quinnat were transferred in September 1985 (A & B), October 1985 (C & D) and November 1985 (E & F).

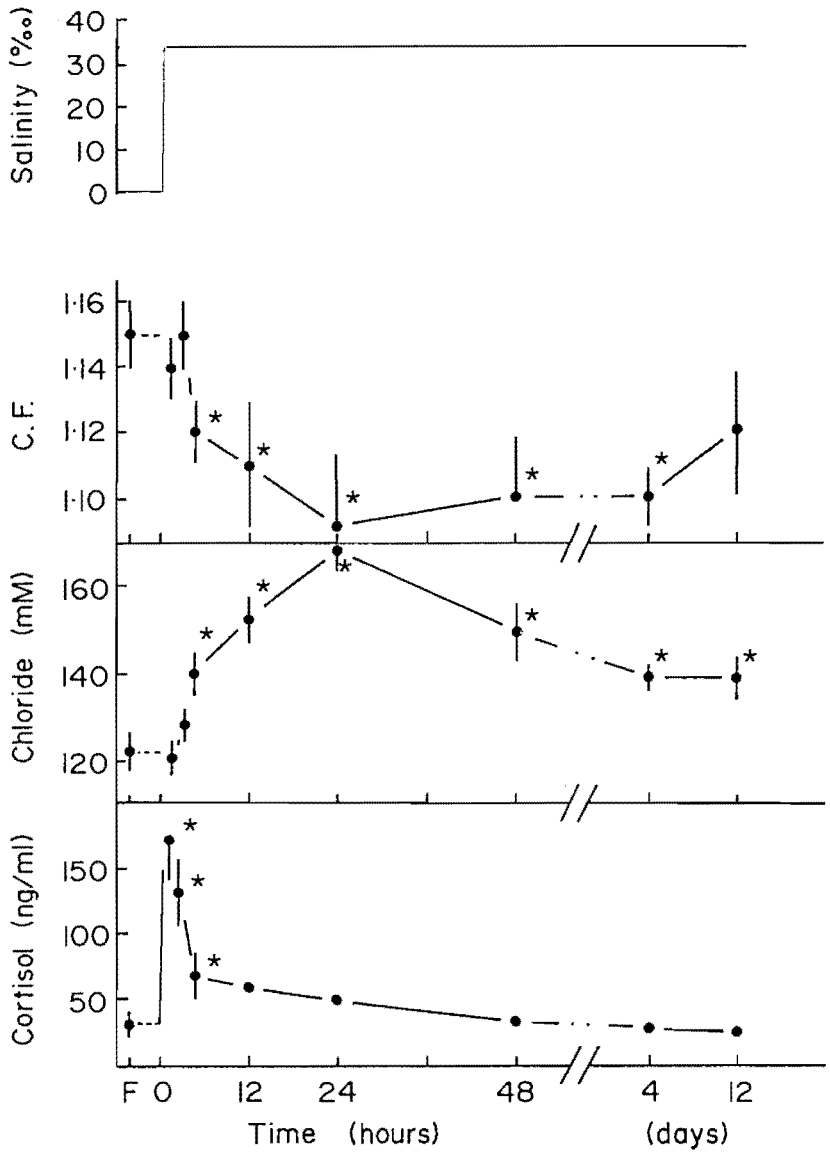

Figure 5. Changes in plasma cortisol, chloride concentration and condition factor (C.F.) of sockeye salmon transferred directly into sea water in November 1985. Results are means±S.E. Asterisks indicate significant difference from the initial freshwater sample (F).

concentration of 168±4 mmol l^{-1} was recorded. Chloride concentrations then decreased and by 4 days had stabilised at 139±5 mmol l^{-1} which was significantly different from basal levels of 122±4 mmol l^{-1} chloride. The condition factor of sockeye salmon significantly decreased after direct seawater transfer and a minimum value of 1.10±0.02 was recorded at 24 hours ($p<0.05$) (Fig. 5). The condition factor remained at approximately this value for the duration of the trial.

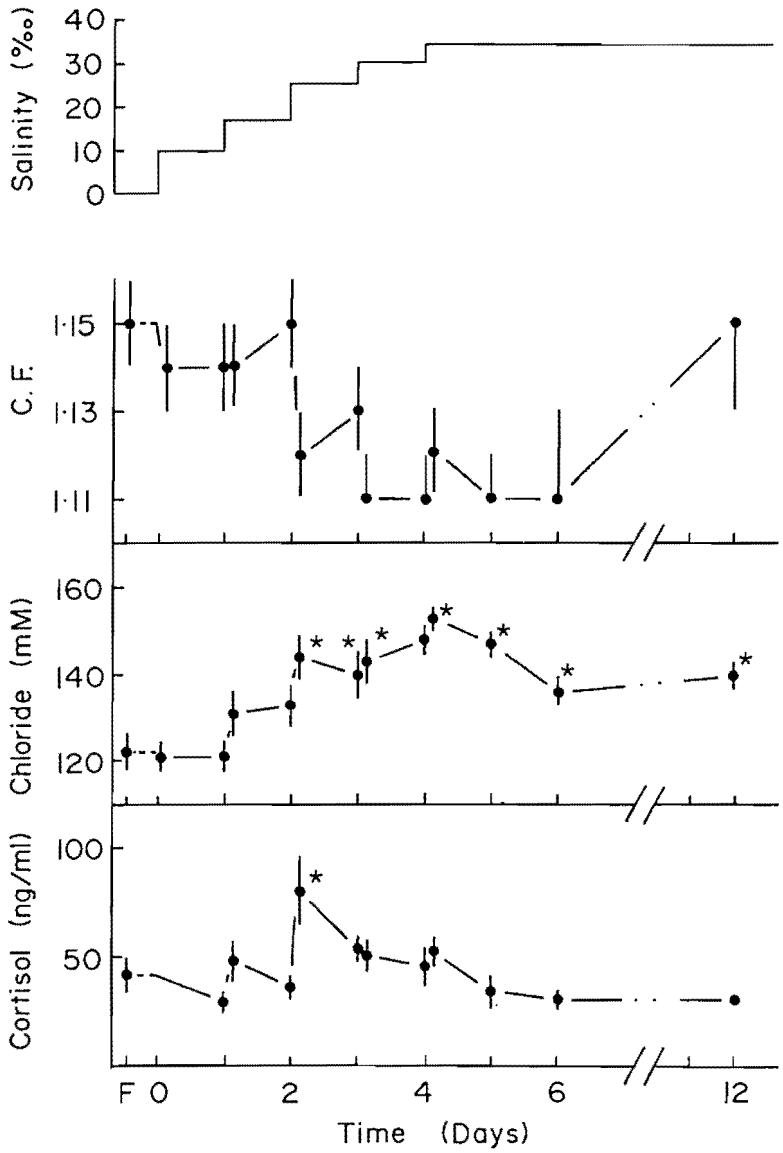

Figure 6. Changes in plasma cortisol, chloride concentration and condition factor (C.F.) of sockeye salmon transferred progressively into sea water in November 1985. Results are means±S.E. Asterisks indicate significant difference from the initial freshwater sample (F).

Fluctuations in plasma cortisol concentrations after the progressive transfer of sockeye salmon were much smaller than changes recorded during their direct transfer (Figs. 5 & 6). A peak in plasma cortisol of 80±16 ng ml^{-1}, was detected 2 hours after the transfer of sockeye from a salinity of 17 ‰ to 25‰ (Fig. 6). This was the only significant change in cortisol concentrations from pre-transfer levels (freshwater adapted sockeye) ($p<0.05$). Plasma chloride concentrations slowly increased after the progressive transfer of sockeye to sea water (Fig. 6). There was no rapid increase as in the direct transfer of sockeye and the magnitude of the increase was also much smaller, rising to 153±3 mmol l^{-1} chloride. Chloride concentrations leveled off at 140±3 mmol l^{-1} 12 days post-transfer. The condition factor of sockeye transferred progressively into

sea water significantly decreased from a pre-transfer value of 1.15±0.01 to 1.10±0.01 after 2 hours in a salinity of 30‰ ($p<0.01$) (Fig. 6). The condition factor returned to pre-transfer levels within 12 days.

Discussion

A gradual increase in salinity over 5 days to full strength sea water did not increase the survival rate of salmon that suffered near to, or 100% mortality after abrupt transfer to sea water. If the salmon failed to adapt to sea water then neither method of seawater transfer was effective in increasing survival. The salmon in these transfers were physiologically unprepared for the transition to sea water. However, one should note that a more gradual increase to 100% sea water (over a period of greater than 5 days) may be needed to stimulate hypoosmoregulatory mechanisms in these fish for an increase in seawater tolerance.

Johnston and Clarke (1985) compared the ionoregulatory ability of rainbow trout (*Salmo gairdneri*) following rapid and slow salinity adaptation. They found that the poorest ionoregulatory capacity occurred in trout directly transferred to sea water and better regulation occurred in trout exposed to a gradual increase in salinity over 22 days. Exposure to a slow increase in salinity is possibly more important for trout, since although there is an increase in salinity tolerance with an increase in size (Bath and Eddy, 1979; Jackson, 1981), trout do not undergo the complex preparatory changes that occur in most Pacific or Atlantic salmon. These developmental modifications (smoltification) allow the salmon to successfully adapt to sea water after an abrupt transfer from freshwater.

Unfortunately, the physiological data collected was from sockeye that successfully adapted to sea water after direct transfer from fresh water. The October 1985 transfer of sockeye salmon could have provided a better understanding of the effect of a gradual salinity increase on the development of osmotic and ionic homeostasis as only a proportion of the salmon directly transferred successfully adapted.

As expected, sockeye salmon progressively transferred to sea water showed a considerably smaller osmotic imbalance than the abruptly transferred sockeye. Plasma chloride concentrations did not increase much above the final chloride

levels that occurred in the sockeye sampled after 7 days in sea water. Plasma cortisol levels also did not rise appreciably in the progressively transferred salmon, although a large, rapid increase in plasma cortisol occurred immediately after sea water entry in the abruptly transferred sockeye. Cortisol has been found to have a dual role in fish, acting as a mineralocorticoid and/or as a glucocorticoid (Chester-Jones et al., 1969). The lack of a large increase in cortisol in the progressively transferred sockeye possibly suggests that the initial rise in cortisol after direct transfer is in response to the transfer acting as a stressor. The direct transfer would be a more severe stressor (causing a larger homeostatic perturbation) than the progressive transfer and so a greater rise in cortisol would be expected. For example, the rise in plasma cortisol may be in response to an increase in buoyancy which would be far more pronounced in the salmon transferred straight into sea water.

In conclusion, sockeye and quinnat salmon are capable of successfully adapting to sea water after an abrupt transfer from fresh water providing that they are physiologically prepared for an existence in sea water prior to transfer (i.e. have undergone smoltification). Therefore, the progressive transfer method is not needed as a technique of increasing survival in these species of salmon unless the criteria suggested above are not met. In cases where sockeye and quinnat are only partially prepared for seawater residence then the exposure to a gradual increase in salinity can increase overall sea water survival.

CHAPTER 8

Surface Ultrastructural Changes in the Gills of Sockeye Salmon During Seawater Transfer: Comparison of Successful and Unsuccessful Seawater Adaptation

Introduction

Gills play a prominent role in maintaining the osmoregulatory balance of teleost fish and this can be attributed to the presence of highly specialised chloride cells that occur within the gill epithelium and which are believed to have ion transporting properties in both hypo-osmotic and hyperosmotic media. In fresh water, the chloride cells actively take up ions from the environment which counteracts the ionic efflux, and in sea water, the cells excrete ions, chiefly sodium and chloride, which counteracts the passive influx of ions (Maetz, 1971; Girard and Payan, 1980; Foskett *et al.*, 1983). Although most teleosts are stenohaline and osmoregulate successfully only in sea water or in fresh water, a few species are euryhaline and are able to tolerate a wide range of salinities. Because of this wide salinity tolerance, many euryhaline species have been used in chloride cell research; e.g. pupfish, *Cyprinodon variegatus* (Karnaky *et al.*, 1976), eels (Thomson and Sargent, 1977), tilapia, *Oreochromis mossambicus* (Hwang, 1987), killifish, *Fundulus heteroclitus* (Hossler *et al.*, 1985), and guppies, *Lebistes reticulatus* (Pisam, *et al.*, 1987). Chloride cells are large columnar-shaped cells that extend through the gill epithelium with their apical surface normally in contact with the external medium. At the ultrastructural level, the chloride cell contains numerous mitochondria that are in close association with an extensive membranous tubular system that is mostly an amplification of the basolateral membrane (Karnaky, 1986; Pisam *et al.*, 1987) . In addition, there is a vesiculotubular system found near the apical surface of the cell (Pisam, 1981).

Viewed with the S.E.M., the teleost gill epithelium can be seen to be covered chiefly by squamous pavement cells which are adorned with surface microridges. Interspersed amongst the pavement cells are chloride and mucous cells. Mucous cells are generally found on the gill filament surfaces, especially the efferent surfaces, whereas chloride cells are most numerous on the afferent filament

surfaces and in the interlamellar spaces (Hossler, *et al.*, 1979; Dunel and Laurent, 1980; Hossler *et al.*, 1985). The structure of the gills and, more particularly, the distribution and the condition of these cell types within the epithelium can give an indication of the physiological state and/or health of the fish (Mallatt, 1985; Evans, 1987). For example, an increase in the number of mucous cells and mucus hypersecretion have been associated with stress resulting from noxious chemicals (Hart and Oglesby, 1979). With respect to the fish's physiological state, the transfer of euryhaline fish from fresh water to sea water has been shown to cause a number of changes in the gill epithelium, especially the reorganisation of chloride cells. An increase in salinity stimulates an increase in the numbers and size of chloride cells and the tubular system of these cells develops into a tight and regular network. There is also an increase in the number of mitochondria and the apical surface becomes invaginated, forming a crypt or pit (Doyle and Epstein, 1972; Sardet *et al.*, 1979; Hossler, 1980; Pisam, 1981; Hwang and Hirano, 1985). It appears that at least in some species these changes are even more complex, as recently Pisam *et al.*, (1987) described in the freshwater-adapted guppy, *Lebistes reticulatus*, two types of chloride cells exist: α- and β- chloride cells. After seawater adaptation only the α- cells remained, and the β- cells degenerated and disappeared.

Most studies have concentrated on changes to the chloride cell during fresh water and sea water adaptation of euryhaline fish, particularly where the transfer has been successful. There has been little published work on the changes in the gill morphology of anadromous salmonids transferred into sea water. Sockeye salmon (*Oncorhynchus nerka*), like all anadromous salmonids, undergo a metamorphosis (smoltification) prior to, or during, seawater migration which assists or enables the salmon to successfully adapt to sea water (for reviews see Hoar, 1976; Folmar and Dickhoff, 1980; Barron, 1986). Failure to adapt to sea water might occur if salmon are transferred before they smoltify or if the fish remain in fresh water too long and revert back to the freshwater form (desmoltify). Other variables such as water temperature, fish health, and salinity may also affect the survival of fish transferred to sea water (Wedemeyer *et al.*, 1980).

The aim of this study is to compare the gill morphologies of sockeye salmon (*Oncorhynchus nerka*) which have successfully and unsuccessfully adapted to sea water. The influence of osmotic stress on the distributions and morphologies of chloride and mucous cells is investigated.

Materials and Methods

Juvenile sockeye salmon were obtained from the Ministry of Agriculture and Fisheries' Glenariffe Hatchery situated on the Rakaia river, and transported to the Edward Percival Field Station, Kaikoura, New Zealand for seawater transfer experiments. Fish were maintained in aerated fresh water (80l tanks) for at least fours days before experimentation. Seawater transfer was achieved by running sea water (34‰) into the tanks at a rate of 10-12 litres/min. The complete exchange of fresh water with sea water took less than twenty minutes. Mortality was recorded and the health of the fish (behavioural observations) also noted. Transfers of sockeye salmon from fresh water to sea water were performed in January, March and July 1986 (See Table 1 for sizes of fish at transfer and seawater and freshwater temperatures). Salmon successfully adapted to sea water in July, but were unsuccessful in acclimating during the January and March seawater transfers. One hundred percent mortality had occurred within five days in January and after four days in March (Fig. 1). For each of the transfers, gills samples were taken from fish in fresh water, and at various stages in sea water. In March, fish were sampled after 48 hours, and in July after 4 and 30 days. In January, samples were taken from seawater transferred salmon that were moribund. These were easily recognised by their dark pigmentation and generally erratic swimming behaviour. Moreover, moribund salmon were frequently seen close to the surface and on occasion, were observed gulping air.

Sampled fish were rapidly anaesthetised in 2-phenoxyethanol (20ml/5l water) and then the first and second gill arches excised, washed in 0.1M cacodylate buffer (pH = 7.3) and fixed in 3% buffered glutaldehyde for 12hrs at 4° C. The gill arches were again rinsed and finally stored in buffer at 4° C.

Scanning electron microscopy

Small numbers of gill filaments (2-6) were dissected from the gill arch and dehydrated in an ethyl alcohol series, followed by an amyl acetate series (two washes in 100% amyl acetate). The filaments were critical point dried with carbon dioxide, mounted onto aluminium stubs and coated with 50nm gold (Polaron E5000). The gill filaments were then examined with an S.E.M. (Cambridge Stereoscan 250). As a measure of relative abundance of cells, the density of both mucous and chloride cells on the afferent surface was determined from the S.E.M. micrographs. Length measurements were taken of the apical surface of the chloride and mucous cells. The greatest linear diameter was used as the

Table 1. Water temperatures and the mean weights of fish transferred directly from fresh water into sea water.

Transfer Date	Fish Weight (g) (x ± S.D.)	Adaptation to Sea Water	Water Temp. (°C) F.W.	S.W.
January 1986	23.1±3.4	Unsuccessful	15.2	17.6
March 1986	58.0±13.2	Unsuccessful	14.2	16.8
July 1986	95.0±13.0	Successful	11.4	10.5

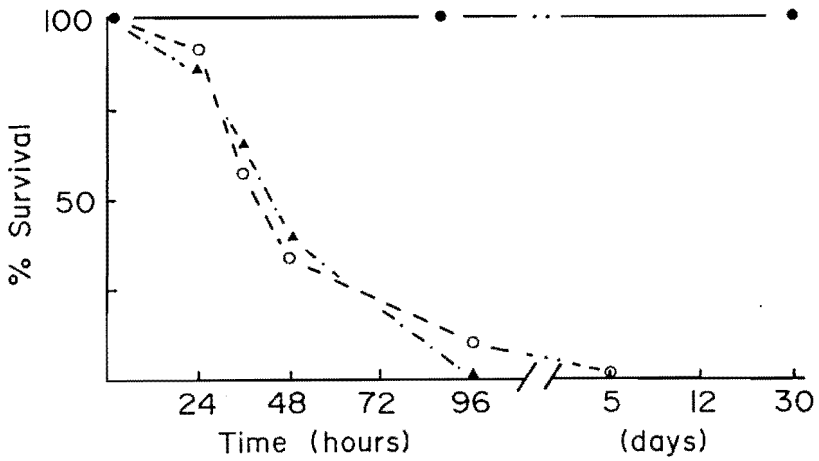

Figure 1. Survival curves for the rapid transfer of sockeye salmon from fresh water to sea water in January (○), March (▲) and July (●).

indicator of cell size.

Light microscopy

Gill filaments stored in buffer were post-fixed in osmium (OsO_4), dehydrated, and then embedded in epoxy resin (Spurrs low viscosity resin). Semi-thick sections were cut, stained with methylene blue and viewed under the light microscope. Quantitative measurements were made of the numbers of mucous cells on the afferent surface in each cross section, and these have been expressed as number of cells per afferent cross-section. The afferent (with respect to blood flow) filamental epithelial surface analysed for such measurements extended from lamella to lamella as indicated in Fig. 2a & b.

Where applicable, results were analysed for significance with ANOVA or the Student's t-test. Results are presented as means ± standard error.

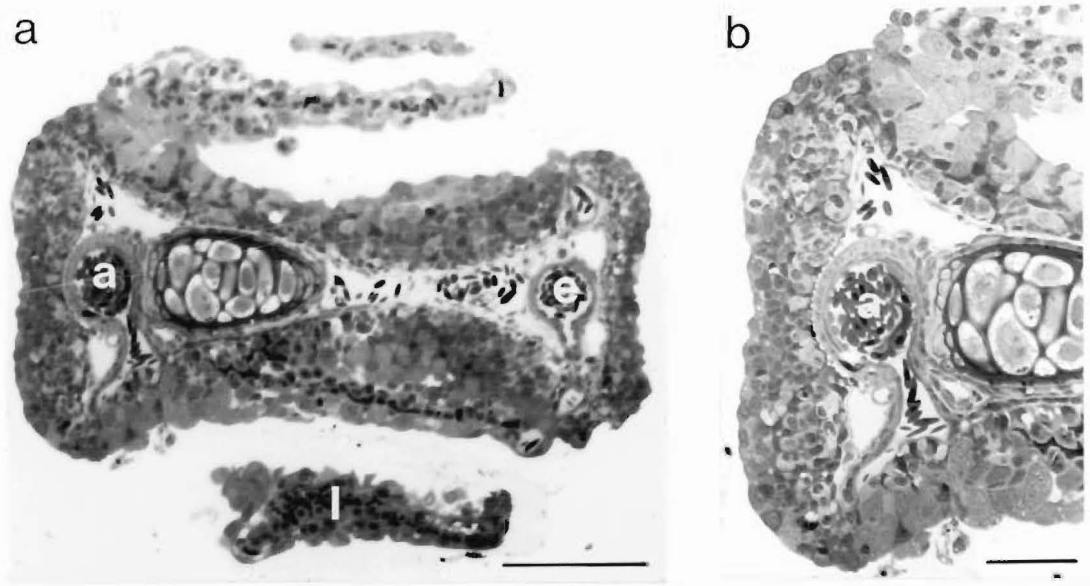

Figure 2a and b. Semi thin cross-sections of a gill filament. (a) afferent artery. (e) efferent artery. (l) lamella. Marked area indicates the resion in which the cell counts were made. Scale bars (a) = 0.1mm (b) = 0.05mm.

Results

The gills of sockeye salmon conformed to the plan seen in most teleosts. A double row of filaments extended from each of the four pairs of gill arches and two stacks of trapezoid shaped respiratory lamellae projected at regular intervals from each of the gill filaments (Fig. 3). The afferent surface of the gill filament had a larger epithelial surface than the efferent surface (Figs. 2a & b and 3). A higher magnification revealed that the surface ultrastructure of the filaments consisted chiefly of squamous epithelial cells (pavement cells) which were covered with microridges (Fig. 4). The continuity of the pavement cells on the afferent surface was interrupted by chloride and mucous cells. No chloride cells were observed on the efferent surface, only pavement and mucous cells. The micrographs (Figs. 4, 5 and 6) are of, and the results below discuss, only the afferent surfaces of the gill filaments.

Freshwater adapted sockeye

In freshwater-adapted sockeye, each chloride cell had a large apical surface that was flat or slightly convex and which was in contact with the environment. The cell's apical surface varied in size, measuring between 4-20µm in length (x=11±2µm). There was variation in the apical surface, and at least two

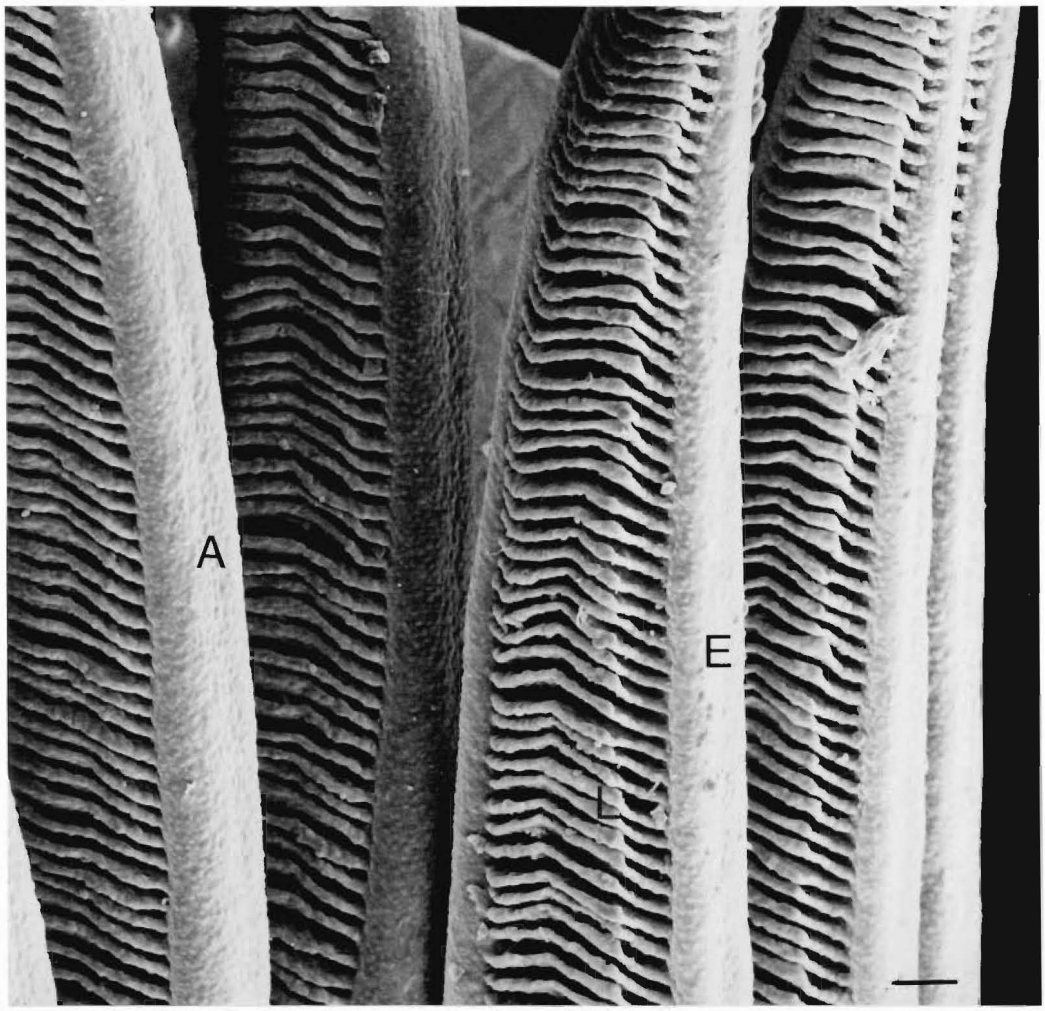

Figure 3. Representatives of gill filaments from the two hemibranchs of a gill arch displaying the afferent (A) and efferent (E) surfaces. (L) respiratory lamellae. Scale Bar = 100µm

morphologically distinct chloride cells could be distinguished (Fig. 4a and 4b). Some chloride cells had an apical surface that was relatively flat and smooth (termed smooth chloride cells), whereas other cells had their exposed surface covered in microvilli to varying degrees (termed rough chloride cells). The coverage by microvilli on individual chloride cells ranged from clearly observed and isolated projections to heavily invaginated surfaces where the microvilli were clustered together at high densities (Fig. 4b). The two cell types were closely associated with each other and often in contact along one cell border.

Table 2. Morphometric data of chloride and mucous cells on the afferent surface of the gills of sockeye salmon. Data is presented as mean±S.D. * indicates a significant difference from the freshwater sample (within one of the monthly transfers) (P<0.05).

Transfer Date	Chloride Cells		Mucous Cells		
	Density (per 1000um^2)	Length of Apical Surface (um)	Density (per 1000um^2)	Length of Apical Surface (um)	No. cells per afferent cross section
July 1986					
F.W.	2.5±0.6	9±2	2.4±0.6	5±2	6.4±1.8
48hrs S.W.	2.6±0.7	6±2*	4.1±0.6*	6±2	6.6±1.7
30d S.W.	1.6±0.3*	1-2 (pits)	3.3±0.6*	6±1*	4.9±1.9
March 1986					
F.W.	1.3±0.4	10±2	2.6±0.9	6±2	5.6±2.4
48hrs S.W.	1.2±0.4	6±1*	4.5±0.5*	11±2*	6.3±2.5
4d S.W.	0.7±0.2*	-	4.5±0.5*	11±2*	6.9±2.4
Jan. 1986					
F.W.	0.9±0.3	13±3	3.9±0.7	9±2	9.9±2.4
S.W. moribund	1.0±0.3	8±2*	6.1±1.6*	10±2	10.3±3.3

The number of chloride cells present on the afferent surface of freshwater adapted salmon varied between the different transfer times (January, March and July 1986) (see Figs. 4a & b, 5a and 6a & b). In July, there was a density of 2.5±0.6 chloride cells per 1000μm^2 which was significantly greater than in the January or March samples ($p<0.01$). The density of chloride cells in January and March were not significantly different from each other, at 0.9±0.3 and 1.3±0.4 cells per 1000μm^2, respectively (Table 2). However, while the apical surfaces of the March and July chloride cells were of similar length (not significantly different), those measured in January were considerably longer than either of the other two months sampled ($p<0.01$ for both March and July). There was also variation between the three freshwater samples of sockeye in the proportion of chloride cells that were of the smooth variety. In January, approximately 80% of the cells had a smooth apical surface, compared to 40% in March and 30% in July.

The number of mucous cells on the afferent surface of freshwater-adapted sockeye was variable (as viewed with the S.E.M.). The mucous cells appeared either as surface swellings (protuberances) as seen in the gills of the March and January salmon (Figs. 5a and 6a & b), or as indentations as in the gills of the January sampled salmon (Fig 5a). A greater density of mucous cells was found in January sampled sockeye salmon (3.9±0.7 cells per 1000μm^2) compared with March and July ($p<0.05$, $p<0.01$, respectively). The size of the cells was also significantly larger in January ($p<0.01$) (Table 2). The quantitative data obtained from gill cross-sections (no. of cells per afferent cross-section, Table 2)

showed a similar trend to the differences that were observed from the S.E.M. measurements, with a greater number of mucous cells per cross-section in January than in March or July ($p<0.01$).

Successful Seawater Adaptation of Sockeye Salmon

The July 1986 sample of sockeye salmon adapted successfully to sea water after direct transfer from fresh water. There was no apparent change in the gross morphology of the gills but, over the time course studied, there were significant changes in the numbers (density and numbers of cells per afferent cross-section) and morphologies of the chloride and mucous cells (Table 2, Figs. 4a-f). After 48 hours in sea water, the density of chloride cells had not changed significantly (Table 2). However, the apical surface length of the cells was markedly reduced, decreasing from a mean freshwater cell length of 9±2µm, to 6±2µm after 48hrs in sea water ($p<0.001$). The margins around the chloride and pavement cells were clearly demarcated and around some chloride cells distinct invaginations were noted (Figs. 4c and 4d). A greater density of mucous cells was seen on the afferent surface (S.E.M.) after 48hrs, increasing from 2.4±0.6 in fresh water to 4.1±0.6 mucous cells per 1000µm^2 in sea water ($p<0.001$). There was, however, no significant increase in the number of mucous cells when cross-sections were viewed (see Table 2). Under the S.E.M., the mucous cells appeared small, and many had not fully 'broken through' the pavement cells, the latter being deformed or stretched as the mucous cells developed beneath them. In these mucous cells, there were no obvious external openings to the surface epithelium. There was a slight increase in the size of other mucous cells which were open to the surface, these increasing from a mean pre-transfer length of 5±2 to 6±2µm after 48 hours ($p<0.05$).

In contrast to the 48 hour samples, after 30 days, no apical surfaces of chloride cells could be seen. These were replaced by pits or crypts (Figs. 4e and 4f). The openings to the pits were 1-2µm in length. The density of chloride cells (1.6±0.3 chloride cells per 1000µm^2) on the afferent filament surface was considerably lower than in the freshwater or 48 hour seawater adapted salmon ($p<0.001$) (Table 2). After 30 days in sea water, mucous cells also occurred at lower densities than at 48hrs post-transfer ($p< 0.01$), but were still higher than the freshwater-adapted sockeye ($p<0.01$) (Table 2). In contrast to the change in density of mucous cells, there was no significant difference in the number of mucous cells per gill cross-section between the freshwater, 48hr and 30 day seawater-adapted sockeye (Table 2). The mean length of the mucous cells at both 48hrs and 30 days, was 6±1µm.

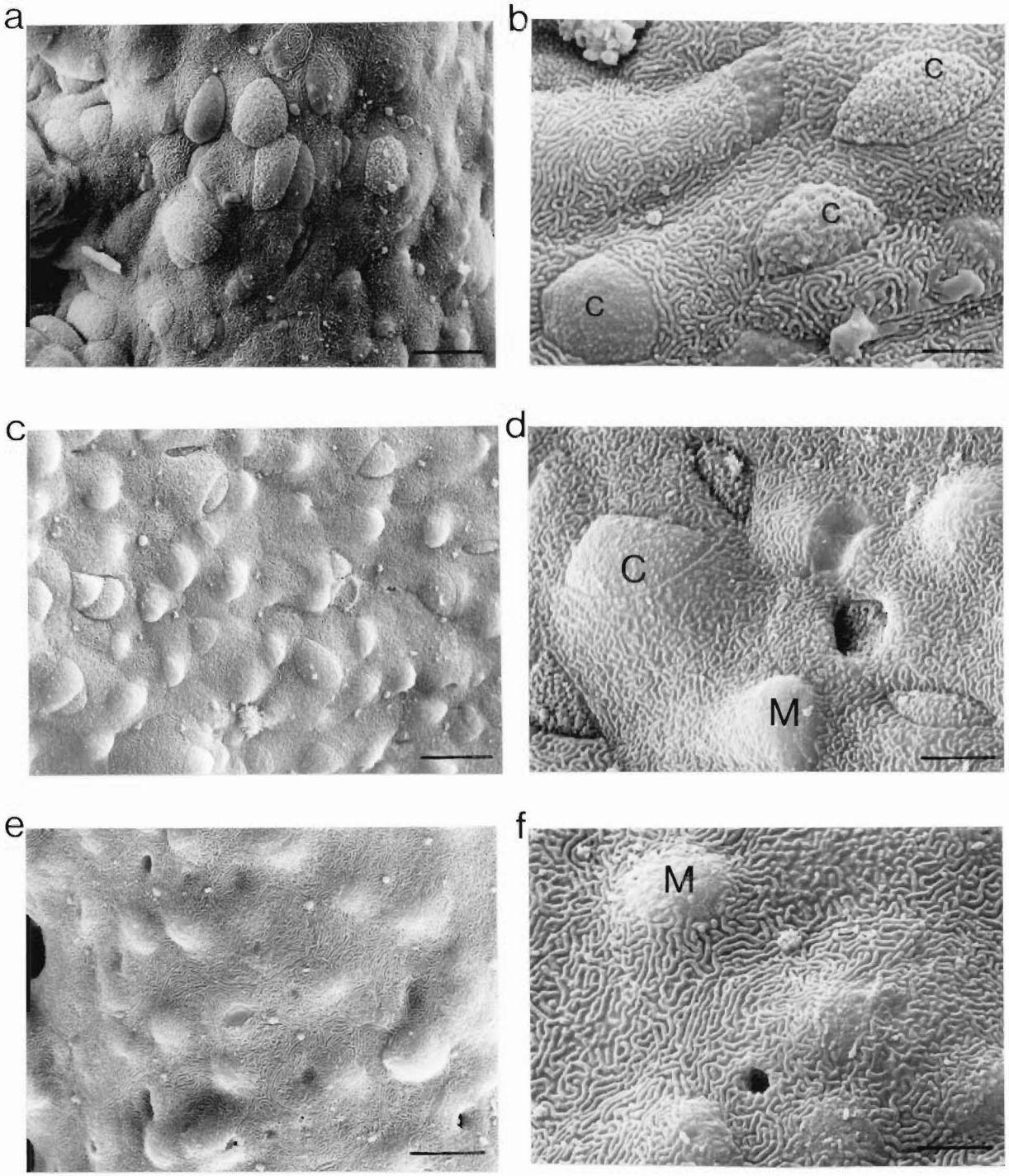

Figure 4. Successful transfer (July) of freshwater-adapted sockeye salmon into sea water. Afferent surfaces of gill filaments of salmon: in fresh water (a & b); after 48hrs in sea water (c & d); and after 30 days in sea water (e & f). (*) chloride cells, (P) pavement cells, (M) mucous cells, arrow - apical crypt of chloride cell. Scale Bars (Figs. a, c & e) = 10μm; (Figs. b, d & f) = 4μm.

Unsuccessful Seawater Transfer of Sockeye salmon

January Transfer

Figures 5a-d are micrographs of the gills of January transferred salmon in which 100% mortality had occurred after 5 days. Gills from freshwater-adapted salmon (Fig. 5a) and from moribund (seawater transferred) salmon (Fig. 5b, c & d) were analysed and gross differences noted. Compared with the freshwater controls, the gill filament stems of the moribund salmon were often shrunken, with ridges and grooves formed along the vertical axis; in addition, their mucous cells had pronounced cell boundaries, as the pavement cells had apparently contracted (Fig 5b). The gills of the January freshwater-adapted salmon had a relatively high density of large mucous cells (3.9±0.7 cells per 1000μm^2, 9±2μm mean length). However, an even higher density of mucous cells occurred in the gill epithelium of the moribund salmon (6.1± 1.6 cells per 1000μm^2, $p<0.01$), and these were also large (10±2μm). However, there was no increase in the number of cells per afferent cross section, nor in the size of the mucous cells of the moribund fish (Table 2). Of all of the gills sampled in this study, the greatest density of mucous cells in the gills occurred in these moribund fish. The mucous cells and their secretions affected a large area of the gill epithelium, often covering a substantial percentage of pavement cells (Fig. 5b, c & d). Chloride cells were smaller in the gills of the moribund salmon compared with the freshwater adapted salmon ($p<0.01$), although there was no change in chloride cell density.

March Transfer

Figures 6a-f are micrographs of the gills of the March salmon in fresh water, and 2 and 4 days after transfer to sea water. Mucous cells increased from a mean density of 2.6±0.9 mucous cells per 1000μm^2 in the gills of freshwater-adapted salmon, to 4.5±1.2 cells per 1000μm^2 after 48hrs in sea water (Table 2, Fig. 6). Also, the size of the mucous cells increased from a mean length of 6±2μm in freshwater, to 11± 1μm after 48hrs ($p<0.01$). The length of the March mucous cells was greater than that of mucous cells formed after 48hrs during the July (successful) transfer (Table 2 $p<0.001$), although the increase in density of mucous cells after transfer to sea water was approximately the same for March and July. After 4 days in sea water, no further change in the size and density of mucous cells was noted. No differences were recorded in the number of mucous cells per afferent cross-section between the freshwater and seawater sampled salmon.

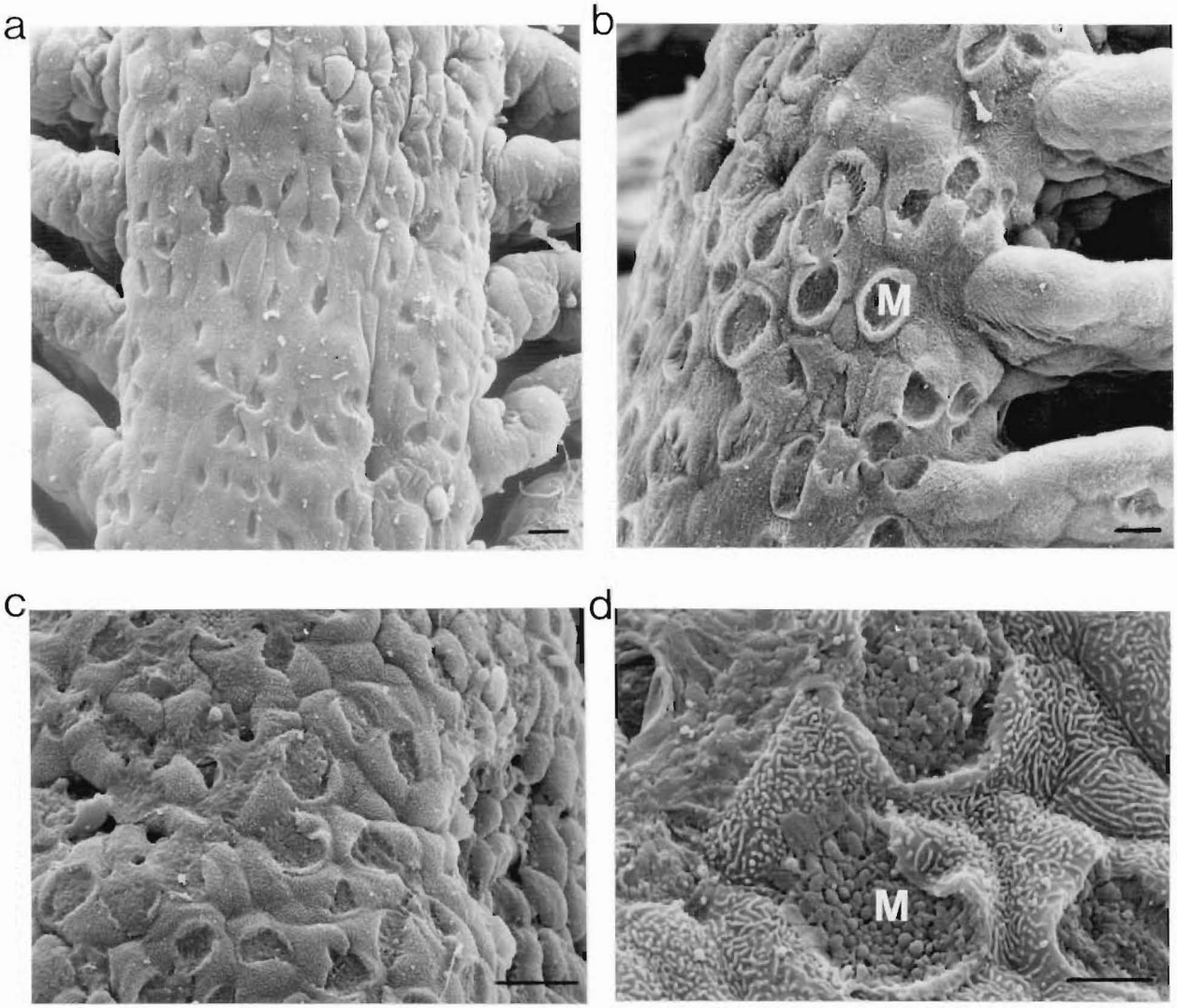

Figure 5. Unsuccessful transfer (January) of freshwater-adapted sockeye salmon into sea water. Afferent surfaces of gill filament of salmon: in fresh water (a); after 4 days in sea water and that are moribund (b, c & d). arrows - chloride cells, (M)- mucous cells. Scale bars (Figs. a & b) = 100µm; (Fig. c) = 10µm; (Fig. d) = 4µm.

In the March transfer, the number of chloride cells on the afferent surface was lower after 4 days, decreasing from 1.3±0.4 in fresh water to 0.7±0.2 cells per 1000µm^2 ($p<0.01$) (Table 2). As in the July salmon transfer, there was also a decrease in the dimensions of the apical surface after the transfer in March ($p<0.001$). The mean length of the apical surface decreased from 10±2 to 6±2µm after 48hrs in sea water.

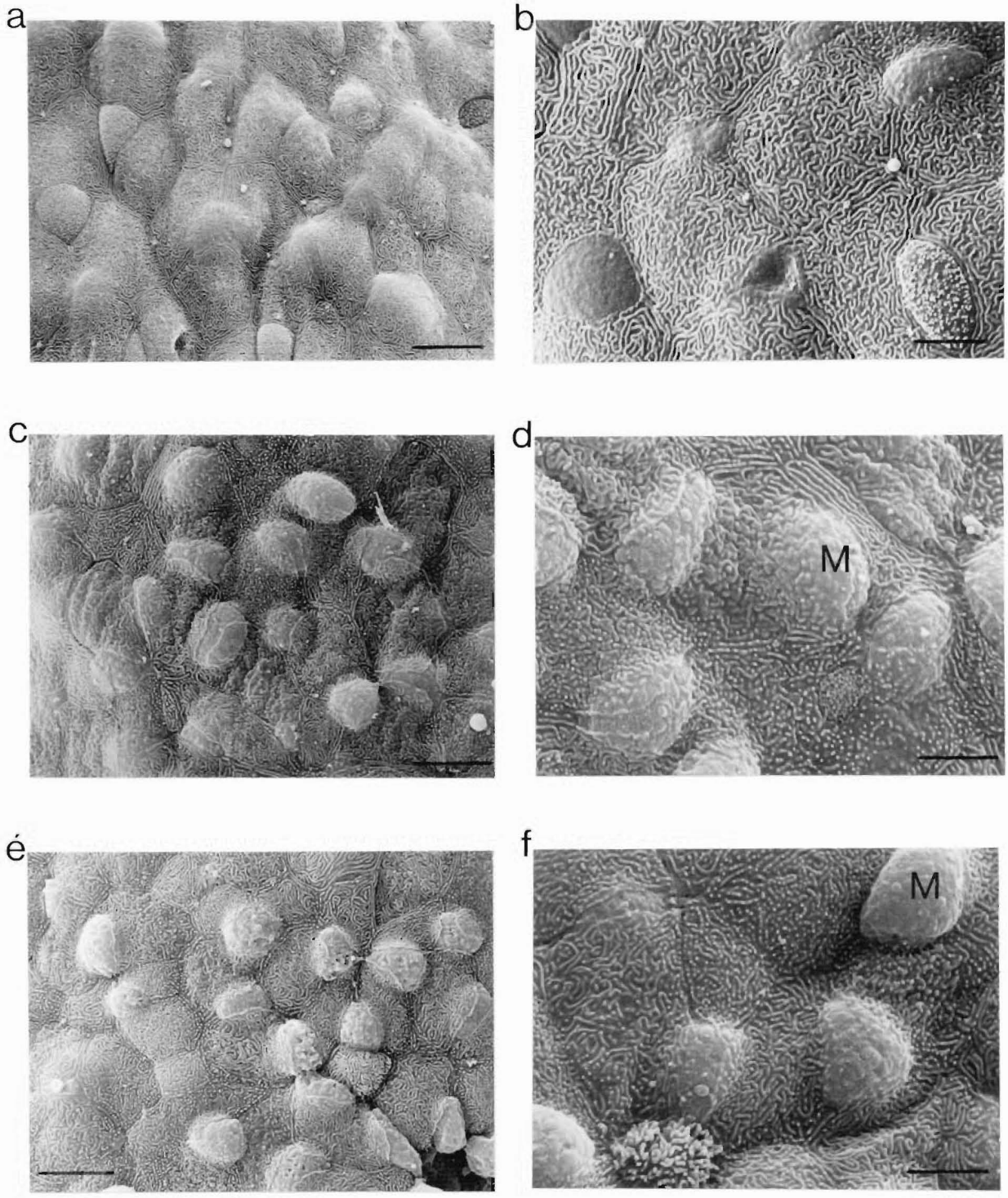

Figure 6. Unsuccessful transfer (March) of freshwater-adapted sockeye salmon into sea water. Afferent surfaces of gill filament of salmon: in fresh water (a & b); after 48hrs in sea water (c & d); and after 4 days in sea water (e & f). Refer to Fig. 3 legend for character descriptions.
Scale bars (Figs. a, c & e) = 10μm; (figs. b, d & f) = 4μm.

Discussion

Sockeye salmon gill ultrastructure, as viewed with the S.E.M., was similar to the pattern seen in the majority of teleosts studied. Pavement cells were the most abundant cell type and their surfaces displayed a complex system of microridges whose primary function is thought to be support of a protective mucus coat (Hossler *et al.*, 1985). Changes in the structure of the gills occurred when salmon were transferred from fresh water to sea water and differences were also noted between gills of salmon that had successfully adapted and those that had unsuccessfully adapted to sea water. Squamous pavement cell surface ultrastructure remained unchanged with the transfer of sockeye from fresh water to sea water. Nevertheless, marked changes were recorded after seawater transfer of sockeye in the frequencies and morphologies of the two other major cell types, the mucous and chloride cells.

Many authors have reported alterations in the structure and numbers of chloride cells in fish after seawater transfer, as well as differences between freshwater-adapted and seawater-adapted fish. Gross changes in seawater transferred fish include an increase in the size and number of chloride cells, and the formation of an apical crypt (Shirai and Utida, 1970; Hossler *et al.*, 1979; Miyamoto *et al.*, 1986). Hossler *et al.* (1985) using the S.E.M., found that in seawater-adapted killifish, the chloride cells occurred at a greater density than in freshwater-adapted killifish and that the cells appeared as deep holes situated along the borders of adjacent pavement cells. For the seawater-adapted sockeye in this study, the chloride cells were visible as holes on the filament surface which opened into apical crypts. However, in contrast to the study of Hossler *et al.* (1985), the density of chloride cells on the afferent filament surface (determined by the S.E.M.) was less than in the freshwater-adapted sockeye. Because only the outer afferent surface can be observed with the S.E.M., it is possible there was an increase in chloride cell numbers in sea water adapted sockeye which would be undetectable with the methods used here. An increased number of chloride cells might be localised to the interlamellar region, or several chloride cells could be associated with each crypt. Neither location can be properly viewed with the S.E.M.. Several studies have shown that the crypt is often shared by two or more chloride cells. At least one of the cells has a fully developed tubular system, while the remaining cell(s) are either similar, or have a less-well developed tubular system. These cells are known as the chloride accessory cells and have been suggested to be the precursors of the chloride cells proper (Ernst *et al.*, 1980;

Hootman and Philpott, 1980; Laurent and Dunel, 1980; Karnaky, 1986).

An increase in chloride cell numbers associated with the successful transfer of fish to sea water (as in the killifish) may have been disguised in sockeye salmon because that increase had already occurred while the sockeye were held in fresh water. The January freshwater-adapted sockeye had low numbers of chloride cells and failed to adapt to sea water. In contrast, the July salmon began with high numbers of chloride cells and were able to adapt to sea water successfully. It appears that a necessary requirement for the successful transfer of sockeye to sea water is a high number of chloride cells initially, a feature that has been described in smoltified salmon (Langdon and Thorpe, 1984). Since salmonids preadapt to the marine environment while in fresh water, one would expect the relative increase in numbers of chloride cells after seawater transfer to be smaller than in species not undergoing this transformation e.g. guppies.

Along with the surface changes in the chloride cell, complex biochemical and ultrastructural changes also occur on transfer of euryhaline teleosts into sea water. The enzyme Na^+-K^+-ATPase, which occurs at high activity in chloride cells, increases when fish are transferred from fresh water to sea water. This has been associated with an increase in chloride cell size, a proliferation in chloride cells and, especially, an amplification of the membraneous tubular system (Karnaky *et al.*, 1976). An increase in gill Na^+-K^+-ATPase activity occurred when sockeye were transferred into, and successfully adapted to, sea water (Chapter 5). This suggests an increase in chloride cell numbers and/or size.

Under the light microscope, the numbers of mucous cells appeared to vary little between the freshwater, and the successful and unsuccessful seawater adapting fish. However, dramatic changes were noted when the gills were examined with the S.E.M. There are several possible explanations for this discrepancy and may be the result of methods used or, more specifically, the limitations of these methods. Several different functional types of mucous cells may exist. The light microscopy analysis of the gills gave very little information on the functional state of the mucous cells whereas the S.E.M. was more useful in determining whether a mucous cell was active on the epithelial surface. Functionally active mucous cells had an obvious opening to the epithelial surface and mucus was present. However, with respect to actual numbers of mucous cells, counts using light microscopy should be more accurate since

surface analysis of the gill epithelium using the S.E.M. would fail to count mucous cells located beneath the pavement cells, the latter perhaps being in an inactive state. This would explain the dramatic increase in density of mucous cells as determined by the S.E.M. without the same magnitudinal increase in numbers of cells per afferent cross-section. These inactive cells could then be rapidly utilised if the situation demands, for example, when the salmon were put into sea water. If just the S.E.M. was used to analyse the gill epithelium, an accurate assessment of the functional number of mucous cells would be obtained, but total numbers of mucous present could be underestimated. This difference would be dependent on the percentage of mucous cells that were functionally inactive and beneath the epithelium; the greater the percentage the more the underestimation. If only the light microscope was used to analyse the gills a more accurate estimate of total numbers of mucous cells would result, but this would not indicate their functional state. Combining S.E.M observations with light microscopy analysis of the gills can give more detailed and accurate information than if only one method of analysis was used.

A prominent feature of the sockeye gills, as viewed with the S.E.M., was the increased density of functional mucous cells after 48hrs in sea water. The increase in mucous cells and hence mucus production would have a protective or preventative role against the influx of ions and loss of water created by direct and rapid transfer into sea water. Ogawa (1974) reported that mucus reduces gill water permeability, and Laurent (1984) suggested that for freshwater fish (which generally have greater numbers of mucous cells) the mucus may control water influx and the loss of ions. A greater osmotic imbalance resulted in the sockeye that failed to adapt to sea water (Chapter 4). In these unsuccessful fish, the mucous cells were larger at 48hrs and 4 days after seawater transfer than the successful sockeye. This greater response to direct seawater transfer may be the result of either the larger osmotic imbalance or a pathological response to the osmotic stress. The greatest number of mucous cells was found in the gills of the moribund fish which tends to support the idea that an increase in numbers is a pathological response. A hypertrophy of mucous cells and an increase in mucus production has been associated with conditions that elicit a generalised stress response in fish. Lethal exposure to contaminants for instance show an especially marked response (Smart, 1981).

Das and Srivastava (1978) and Zacone (1981) reported that mucous cells were able to transform into chloride cells, although Laurent (1984) suggested that this

needs to be substantiated by further studies. In the salmon that failed to adapt to sea water, chloride cells were conspicuously low in density on the afferent surface. The initial response in these fish might have been increased mucus production, thereby preventing water loss and ion influx, rather than actively compensating for these movements.

The January sampled, freshwater-adapted sockeye had the highest density and cross-sectional numbers of mucous cells compared with the other freshwater sampled salmon. This could be the result of January's warmer water temperatures eliciting a generalised stress response in the form of increased mucus production on epithelial surfaces. Diffusion rates in water are faster at higher temperatures and the increased mucus production might be advantageous in reducing the outflux of ions and the influx of water. Supporting this hypothesis is the mortality rate of the January and March seawater-transferred salmon. In the absence of mucus one would expect the fastest mortality to occur in the warmest water. However, the results show that 100% mortality occurred after 4 days in March (water temperature = 16.8 °C) compared with 5 days in January (water temperature = 17.6 °C). The increased mucus production in the January sockeye might have been a factor that prolonged survival.

Apical morphology of chloride cells in freshwater-adapted sockeye was varied. The surface could be either smooth, or invaginated by microvilli. In the freshwater-adapted guppy, Pisam (1987) found the presence of two types of chloride cells which had, among other characteristics, differing apical morphologies. The α- chloride cells had an apical surface that was smooth, whereas the β- cells had a surface that was bumpy and invaginated due to the existence of a well developed vesiculo-tubular system just below the apical membrane. The chloride cells seen in sockeye might equate to the α- and β- cells found in the guppy. With such clear differentiation in their morphologies, functional differences between the smooth and invaginated chloride cells are likely. Since teleostean chloride cells play an important role in ionic and osmotic regulation (Karnaky, 1986), the distribution and the ratio of smooth and invaginated chloride cells may reflect the osmoregulatory status of the salmon, especially with respect to the freshwater osmoregulatory changes associated with smoltification. Variation occurred in the ratios of smooth to invaginated chloride cells. A high proportion of smooth cells occurred in the freshwater-adapted January salmon that failed to go into seawater, whereas in the July freshwater-adapted salmon that successfully transferred, there was a

higher proportion of invaginated cells. In March there was an equal proportion of invaginated and smooth chloride cells. From these observations it would appear that in salmon at least, a greater proportion of invaginated chloride cells are required for successful seawater adaptation. Further investigations are needed to help clarify the specific function of these different types of chloride cells.

CHAPTER 9

Conclusions

The purpose of this study was to examine the seawater adaptability of New Zealand's quinnat and sockeye salmon which were introduced from North America about 90 years ago. In investigating this topic it was neccessary to acquire an understanding of the factors, both environmental and physiological which affected the seawater tolerance of these salmon. This study focused on the physiological changes occurring in smoltifying freshwater-adapted salmon, and also in salmon transferred to sea water. These changes were discussed with respect to physiological stress.

Despite the wealth of information on the seawater adaptability of Pacific salmon resident in North America (Bern and Mahnken, 1982; Thorpe et al., 1985; Hoar, 1988) research on this topic in New Zealand is limited. The fish stocks in New Zealand and environmental conditions present a unique situation that can not be compared with the North American scenario. For example, sockeye salmon which originally came from an anadromous population have formed a totally fresh water population in New Zealand and there was some doubt over whether or not these salmon could survive and grow in sea water. With respect to environmental conditions and in particular water temperatures, New Zealand has water temperatures that are considerably warmer than those found in the North American habitat. The New Zealand temperatures are well above the upper limits of most Nothern Hemisphere studies. To date there have been no studies which have looked at the success of, and the physiological changes in salmon transferred to sea water that is as high as 19°C, which is the of normal summer sea water temperatures around the northern parts of the South Island of New Zealand.

In part this study was designed to have practical applications for the aquaculture of salmon. Hence, the transfer methods used, placing the salmon directly from fresh water into sea water are based on methods that are used in sea-cage rearing operations. This type of transition into sea water is unlikely to happen in wild stocks of salmon as they have the capability of choosing the time to migrate down to the sea and once there can remain in the intermediate salinities found in estuaries and around river mouths.

For the ease of this discussion, sockeye and quinnat salmon will not be differentiated as there was very little difference between the seawater adaptability of the two species. Sockeye despite being voluntarily landlocked are able to successfully adapt and grow in sea water.

The findings of this study can be broadly divided into the factors influencing seawater survival in salmon, and indicators of seawater survival. The former relates to the actual ability of the fish to survive a rapid change from fresh water to sea water, while the latter relates to the ability of the aquaculturist to predict the success of a transfer.

The factors affecting the seawater survival of salmon can be be conveniently separated into external influences (such as the method of transfer of salmon to sea water and seawater temperatures), and inherent changes in the salmon which lead to increases in their hypoosmoregulatory ability. It should be noted however, that the salmon's hypoosmoregulatory development in fresh water is also affected by environmental conditions (Hoar, 1988).

An increase in salinity tolerance occurred with an increase in the size of the salmon. This reflects the effect of a decrease in surface area to volume (as the salmon gets larger) on ion and water movements. Further increases in salinity tolerance occurred in the salmon that could not be related to just size alone, but to a change in the physiological state of the freshwater-adapted fish. The physiological changes prepared the salmon for the transition into sea water. This process, known as the parr-smolt transformation or smoltification is a critical process which can be considered as a major factor influencing the seawater adaptability of salmon. Hence, many indicators of seawater survival are based on determining when the parr-smolt transformation occurs. A critical size needs to be reached before the parr-smolt transformation can occur and this metamorphosis is activated by seasonal changes such as an increase in the photoperiod (Bern and Mahnken, 1982; Thorpe et al., 1985; Hoar, 1988).

In this study the salinity tolerance of the salmon fluctuated seasonally, increases in seawater adaptability occurring in spring and in late autumn/early winter. Coinciding with the increases in salinity tolerance were elevated activity levels of the gill Na^+-K^+-ATPase enzyme. High levels of this enzyme in freshwater-adapted salmon have been associated with the parr-smolt transformation (Folmar and Dickhof, 1978; 1980; 1981; Hoar, 1988). With the onset

of summer and high temperatures there was a decrease in the activity of this enzyme, which indicated that the salmon were desmoltifying. Salmon were not able to survive the transition during this time. This decrease in activity may be due either to an endogenous cyclical nature of the enzyme (Zaugg, 1982; Virtanen and Soivio, 1985; Folmar and Dickhoff, 1979. 1980, 1981) and/or a direct effect of the increasing water temperatures. High freshwater temperatures have been shown to have an inhibitory effect on the development of the gill Na^+-K^+ ATPase activity in freshwater adapted salmon (Zaugg, 1981; Hoar, 1988) and may also account for the desmoltification process. There is a corresponding increase in diffusion rates with an increase in temperature (Prosser, 1973) so during the summer one would expect a greater flux of ions in and out of the salmon. High activities of gill ATPase (late autumn) are related to low plasma ion concentrations in salmon which are still resident in fresh water, even though the water temperatures are low. Higher temperatures in the summer and the resulting increase in fluxes could mean that these fish are unable to maintain high ATPase activities whilst still in fresh water. Hence the ability to move into sea water is lost. It would be interesting to test this assumption by placing salmon with high ATPase activities into warmer fresh water and noting the changes in plasma ionic concentration and ATPase activity levels. I would predict very low ion levels in these salmon and a decrease in ATPase activity.

Generally salmon that have smoltified will successfully transfer to sea water. However, external conditions can overrride this preparatory process and result in mortality of salmon on transfer. High seawater temperatures (19°C) were found to decrease the success of seawater transfer of salmon (see Chapter 6).

As a method of increasing seawater survival, exposing salmon to a gradual increase in salinity does not appear to be of any advantage. If salmon are correctly diagnosed of being capable of surviving the seawater transition, then the direct seawater transfer method is more than adequate.

As mentioned, smoltification is a major factor determining the success of the seawater transfer of salmon. Predictors or indices of successful seawater transfer of salmon are based on determining when the salmon smoltify but also have to take into account environmental conditions. This is especially relevant with respect to New Zealand conditions. The developmental stage (pre-smoltified, smoltified, desmoltified) of salmon and hence their ability to survive transfer to sea water is in a continual state of flux. Therefore it is

difficult to determine when the salmon are maximally prepared for the transition into sea water (fully smoltified). However, seawater transfer at this 'peak' in hypoosmotic capability is not required for success seawater adaptation in the salmon. The salmon need only to reach a certain level of hypoosmotic ability (a threshold) for seawater adaptation to be successful. This threshold level can increase or decrease depending upon environmental conditons. The salmon transferred into warm (19°C) sea water need to undergo more extensive preparatory modifications than salmon transferred to colder (13°C) sea water. Therefore, if indicators of salmonid seawater survival obtained from freshwater-adapted salmon are to be reliable then the environmental conditions must be qualified. For example, the predictors may only be reliable if the seawater temperature in which they are to be transferred is below 15°C. Monitoring physiological variables in salmon following the transfer into sea water would however, overcome this problem of having qualifiers, provided that the test transfer is made in seawater that is representative of the sea water conditions found around the sea-cage rearing sites. This would give a direct and more accurate indication of the hypoosmotic regulatory ability of the salmon. Chapter 5 discusses specific indicators in more detail.

The practicality of the methods to determine the hypoosmoregualtory status of the salmon is of importance to the salmon farm operator. Gill Na^+-K^+-ATPase activity is an accurate indicator of smoltification but the assay is lengthy and much equipment is needed. Similarly, for plasma cortisol determination, specialised equipment is required. Without comprising accuracy, the estimation of either the salmon's plasma chloride or sodium concentration after 24 hours in sea water would seem to be the most practicable. Only one piece of specialised equipment is needed, either a flame photometer or a chloride meter.

Finally, the underlying theme of this study was the concept of physiological stress. Both smoltification and the direct transfer of salmon from fresh water to sea water were viewed as potential stressors that could elicit a corresponding physiological stress response from the fish. Plasma cortisol was used as the major and primary indicator of a stress response although its role in both smoltification and seawater adaptation could be as a mineralocorticoid, and not as a glucocorticoid. I believe smoltified salmon (having high gill Na^+-K^+-ATPase activities) are physiologically stressed while they remain in fresh water as their osmoregulatory mechanisms are modified for their future life in sea water and so are not totally designed for functioning efficiently in fresh water. Desmoltification supports this argument, as if the salmon were not

stressed then it would seem reasonable for them to remain in the smoltified state. In addition, smoltified salmon have a preference for sea water (Hoar, 1988). So in effect, it is not the process of smoltification that is the stressor but as a consequence of this transformation fresh water becomes a stressor to the salmon. In salmon that are transferred directly to sea water, it is abrupt change of salinity that is the stressor, although the severity of sea water as a stressor is dependant of the ability of the salmon to adapt to this new medium. The salmon that failed to adapt to sea water showed a greater stress response.

Acknowledgements

I gratefully thank my supervisors Drs. Bill Davison and Malcolm Forster for their encouragement and excellent advice in all facets of this study. Their experience and appreciation of fish physiology provided me with an excellent grounding and source of knowledge. A special thanks to Bill who broadened my fish physiology horizons during my Ph.D. with the welcomed excursions to Antarctica.

I thank Roy Thompson and his technical staff who provided the much appreciated assistance and expertise that could seemingly find a solution to any problem that happened to present itself. A special mention must go to Jack Van Berkel the manager of the Edward Percival Field Station who persevered with the monthly job of collecting fresh water and who made my visits to the lab one of the highlights of this study. Many thanks go to Jan McKenzie whose dexterity with knobs on the S.E.M. is second to none. I thank Terry Williams for photographic assistance.

Salmon were supplied by F.R.D. (M.A.F.) and collected from the Glenariffe salmon hatchery. Many thanks goes to Dr. Peter Todd, Ceri Hopkins and Mike Field-Dodgson, staff at F.R.D. Kyle St. Fish were also supplied by Dr Ray French and Southland Salmon and both provided me with a knowledge of the logistics and problems associated with the sea-cage rearing of salmon.

With the final preparation of this thesis, I gratefully thank Tracey Robinson who printed out the final copy and added a personal touch to the ^{o}C, $\pm$, and the $‰$ s. My deepest appreciation must go to Carol Bremner and Sarah Sweet who collated and typed the reference list. A very special thanks go to my fellow physiology Ph.D. student and good friend, Fran Waldron who gratefully proof read the entire final draft of this thesis and who provided me with the much needed coffee breaks and stimulating idle chatter.

Personal funding was provided by a U.G.C. scholarship and research costs met in part by Southland Salmon.

Finally I would like to thank my friends, fellow Ph.D students (Peter Carey, Simon Pollard and Joseph Waas) and family who persevered with my pessimism and supported me throughout this study.

References

Adedire, C.O. and Oduleye, S.O. (1984) Stress-induced water permeability changes in the tropical cichlid, *Oreochromis niloticus* (Trewavas). Journal of Fish Biology, 25, 463-471.

Barron, M.G. (1986) Endocrine control of smoltification in anadromous salmonids. Journal of Endocrinology, 108, 313-319.

Barton, A., Schreck, C.B., Ewing, R.D., Hemmingsen, A.R. and Patino, R. (1985) Changes in plasma cortisol during stress and smoltification in coho salmon, *Oncorhynchus kisutch.* General and Comparaive Endocrinology, 59, 468-471.

Barton, B.A. and Peter, R.E. (1982) Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia, and cold shock. Journal of Fish Biology, 20, 39-51.

Barton, B.A., Peter, R.E. and Paukencu, C.R. (1980) Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Canadian Journal of Fisheries and Aquatic Sciences, 37, 805-811.

Barton, B.A., Schreck, C.B. and Sigismondi, L.A. (1986) Multiple acute disturbances evoke cumulative physiological stress responses in juvenile chinook salmon. Transactions of the American Fisheries Society, 115, 245-251.

Barton, B.A., Weiner, G.S. and Schreck, C.B. (1985) Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. Canadian Journal of Fisheries and Aquatic Sciences, pp 710-717.

Bath, R.N. and Eddy, F.B. (1979) Salt and water balance in rainbow trout (*Salmo gairdneri*) rapidly transferrd from fresh water to sea water. Journal of Experimental Biology, 83, 193-202.

Battram, J.C. (1987) Chloride uptake in the gills of the fresh-water-adapted brown trout (*Salmo trutta*). Journal of Comparative Biochemistry and Physiology, 86A, 245-249.

Beggs, G.L., Holeton, G.F. and Crossman, E.J. (1980) Some physiological consequences of angling stress in muskellunge, *Esox masquinongy* Mitchill. Journal of Fish Biology, 17, 649-659.

Bern, H.A. and Mahnken, C.V.W. (1982) Salmonid smoltification. Aquaculture, 28, 1-270.

Binoux, M.A. and Odell, S.E. (1973) Separation methods using charcoal. Journal of Clinical Endocrinology and Metabolism, 36, 303-313.

Blackburn, J. and Clarke, W.C. (1987) Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1515 35pp.

Boeuf, G. and Harache, Y. (1982) Criteria for adaptation of salmonids to high salinity seawater in France. Aquaculture, 28, 163-176.

Boeuf, G., Lasserre, P. and Harache, Y. (1978) Osmotic adaptation of *Oncorhynchus kisutch* Walbaum II. Plasma osmotic and ionic variations, and gill Na^+-K^+-ATPase activity of yearling coho salmon transferred to sea water. Aquaculture, 15, 35-52.

Boeuf, G. and Prunet, P. (1985) Measurements of gill (Na^+-K^+)-ATPase activity and plasma thyroid hormones during smoltification in Atlantic salmon (*Salmo salar L.*). Aquaculture, 45, 111-119.

Boeuf, G., Le Roux, A., Gaignon, J.L. and Harache, Y. (1985) Gill (Na^+-K^+)-ATPase activity and smolting in Atlantic salmon (*Salmo salar L.*) in France. Aquaculture, 45, 73-81.

Brett, J.R. (1952) Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. Journal of the Fisheries Research Board of Canada, 9, 265-323.

Bry, C. (1981) Daily variations in plasma cortisol levels of individual, female rainbow trout (*Salmo gairdneri* Richarson): Differences between normal and non-adapted fish. In Stress and Fish (A.D. Pickering, ed.), pp 325-326. Academic Press, New York/London.

Buckman, M. and Ewing, R.D. (1982) Relationship between size and time of entry into the sea and gill (Na^+-K^+)-ATPase activity for juvenile spring chinook salmon. Transactions of the American Fisheries Society, 111, 681-687.

Burton, M.P. and Idler, D.R. (1984) Can Newfoundland landlocked salmon *Salmo salar L.*, adapt to sea water? Journal of Fish Biology, 24, 59-64.

Butler, P.J., Taylor, E.W., Capra, M.F. and Davison, W. (1978) The effect of hypoxia on the levels of circulating catecholamines in the dogfish *Scyliorhinus canicula*. Journal of Comparative Physiology, 127, 325-330.

Carl, L. and Healey, M.C. (1984) Differences in enzyme frequency and body morphology among three juvenile life history types of chinook salmon (*Oncorhynchus tshawytscha*) in the Nanaimo River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences, 41, 1070-1077.

Carmichael, G.J., Tomasso, J.R., Simco, B.A. and Davis, B. (1984) Characterization and alleviation of stress associated with hauling largemouth bass. Transactions of the American Fisheries Society, 113, 778-785.

Carmichael, G.J., Wedemeyer, G.A., McCraren, J.P. and Millard, J.L. (1983) Physiological effects of handling and hauling stress on smallmouth bass. Progressive Fish Culturist, 45, 110-113.

Chakraborti, P.K., Weisbart, M. and Chakraborti, A. (1987) The presence of corticosteroid receptor activity in the gills of the brook trout, *Salvelinus fontinalis*. General and Comparative Endocrinology, 66, 323-332.

Chernitsky, A.G. (1980) Functional state of chloride cells of Baltic salmon (*Salmo salar* L.) at different stages of its life cycle. Comparative Biochemistry and Physiology, 67A, 519-522.

Chester-Jones, I., Chan, D.K.O., Henderson, I.W. and Ball, J.N. (1969) The adrenocortical steriods, adrenocorticotropin and the corpuscles of Stannius. In Fish Physiology, II (W.S. Hoar and Randall, eds.) London and New York: Academic Press 322-376.

Clarke, W.C. (1982) Evaluation of the seawater challenge test as an index of marine survival. Aquaculture, 28, 177-183.

Clarke, W.C. and Nagahama, Y. (1977) Effect of premature transfer to sea water on growth and morphology of the pituitary, thyroid, pancreas, and interrenal in juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Zoology, 55, 1620-1630.

Clarke, W.C. and Shelbourn, J.E. (1985) Growth and development of seawater adaptability by juvenile fall chinook salmon (*Oncorhynchus tshawytscha*) in relation to temperature. Aquaculture, 45, 21-31.

Clarke, W.C., Shelbourn, J.E. and Brett, J.R. (1978) Growth and adaptation to sea water in "underyearling" sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon subjected to regimes of constant or changing temperature and day length. Canadian Journal of Zoology, 56, 2413-2421.

Clarke, W.C., Shelbourne, J.E. and Brett, J.R. (1981) Effect of artifical photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho, chinook and sockeye salmon. Aquaculture, 22, 105-116.

Conte, F.P. and Wagner H.H. (1965) Development of osmotic and ionic regulation in juvenile steelhead trout *Salmo gairdneri*. Comparative Biochemistry and Physiology, 14, 603-620.

Dadswell, M.J., Klauda, R.J., Moffitt, C.M., Saunders, R.L., Rulifson, R.A. and Cooper, J.E. (1987) Common Strategies of Anadromous and Catadromous Fishes. American Fisheries Society Symposium 1, 561pp.

Das, S. and Srivastava, G.J. (1978) Responses of gill to various changes in salinity in fresh water teleost *Colisa fasciatus* (Bl. and Schn.). Z. mikrosk-anat. Forsch, 92, 770-780.

De Renzis, G. and Bornancin, M. (1984) Ion transport and gill ATPases. In Fish Physiology, 10B, 65-104.

DeVries, A.L. and Ellory, J.C. (1981) The effect of stress on ion transport in fish erythrocytes. Journal of Physiology, 324, 51pp.

Dickhoff, W.W., Folmar, L.C. and Gorbman, A. (1978) Changes in plasma thyroxine during smoltification of coho salmon, *Oncorhynchus kisutch.* General and Comparative Endocrinology, 36, 229-232.

Dickhoff, W.W., Folmar, L.C., Mighell, J.L. and Mahnken, V.W. (1982) Plasma thyroid hormones during smoltification of yearling and underyearling coho salmon and yearling chinook salmon and steelhead trout. Aquaculture, 28, 39-48.

Dickhoff, W.W., Sullivan, C.V. and Mahnken, C.V.W. (1985) Thyroid hormones and gill ATPase during smoltification of Atlantic salmon (*Salmo salar*) Aquaculture, 45, 376.

Donaldson, E.M. (1981) The pituitary-interrenal axis as an indicator of stress in fish. Stress and Fish (A.D. Pickering ed.) London New York: Academic Press 11-48.

Donaldson, E.M. and Dye, H.M. (1975) Corticosteroid concentrations in sockeye salmon (*Oncorhynchus nerka*) exposed to low concentrations of copper. Journal of the Fisheries Research Board of Canada, 32, 533-539.

Doyle, W.L. and Epstein, F.H. (1972) Effects of cortisol treatment and osmotic adaptation on the chloride cells in the eel *Anguilla rostrata.* Cytobiologie, 6, 58-73.

Dunel-Erb, S. and Laurent, P. (1980) Ultrastructure of marine teleost gill epithelia: SEM and TEM study of the chloride cell apical membrane. Journal of Morphology 165, 175-186.

Elliott, J.M. (1981) Some aspects of thermal stress on freshwater teleosts. In Stress and Fish (A.D. Pickering, ed.) London and New York: Academic Press, 209-245.

Epstein, F.H., Cynamon, M. and McKay, W. (1971) Endocrine control of Na^+-K^+-ATPase and seawater adaptation in *Anguilla rostrata.* General and Comparative Endocrinology, 16, 323-328.

Epstein, F.H., Katz, A.I. and Pickford, G.E. (1967) Sodium and potassium-activated adenosine triphosphatase of gills: Role in adaptation of teleosts to salt water. Science, 156, 1245-1247.

Ernst, S.A., Dodson, W.C., and Karnaky, Jr., K.J. (1980) Structural diversity of occluding junctions in the low-resistance chloride secreting opercular epithelium of seawater-adapted killifish (*Fundulus heteroclitus*). Journal of Cell Biology, 87, 488-497.

Evans, D.H. (1984) The roles of gill permeability and transport mechanisms in euryhalinity. Fish Physiology, pp 239-283.

Evans, D.H. (1987) The fish gill: site of action and model for toxic effects of environmental pollutants. Environmental Health Perspectives, 71, 47-58.

Ewing, R.D. Hemmingsen, .R., Evenson, M.D. and Lindsay, R.L. (1985) Gill (Na^+K^+)-ATPase activity and plasma thyroxine concentrations do not predict time of release of hatchery coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) for maximum adult returns. Aquaculture, 45, 359-373.

Finstad, B., Staurnes, M. and Reite, O.B. (1988) Effect of low temperature on sea-water tolerance in rainbow trout, *Salmo gairdneri.* Aquaculture, 72, 319-328.

Flain, M. (1981) The history of New Zealand's salmon fishery. Proceedings of the Salmon Symposium. Fisheries Research Divison Occasional Publication No 30, 8-10.

Folmar, L.C. and Dickhoff, W.W. (1978) Some physiological changes during smoltification of coho salmon (*Oncorhynchus kisutch*). American Zoologist, 18, 606.

Folmar, L.C. and Dickhoff, W.W. (1979) Plasma thyroxine and gill Na^+-K^+ ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch.* Comparative Biochemistry and Physiology, 63, 329-332.

Folmar, L.C. and Dickhoff, W.W. (1980) The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. Aquaculture, 21, 1-37.

Folmar, L.C. and Dickhoff, W.W. (1981) Evaluation of some physiological parameters as predictive indices of smoltification. Aquaculture, 23, 309-324.

Folmar, L.C., Dickhoff, W.W., Mahnken, V.W. and Waknitz, F.W. (1982) Stunting and parr-reversion during smoltification of coho salmon (*Oncorhynchus kisutch*) Aquaculture, 28, 91-104.

Fontaine, M. (1975) Physiological mechanisms in the migration of marine and amphihaline fish. Advances in Marine Biology, 13, 241-355.

Foskett, J.K., Bern, H.A., Machen, T.E. and Conner, M. (1983) Chloride cells and the hormonal control of teleost fish osmoregulation. Journal of Experimental Biology, 106, 255-281.

Fouchereau-Peron, M., Arlot-Bonnemains, Y., Moukhtar, M.S. and Milhaud, G. (1986) Adaptation of rainbow trout (*Salmo gairdneri*) to sea water: changes in calcitonin levels. Comparative Biochemistry and Physiology, 83A, 83-87.

Girard, J.P. and Payan, P. (1980) Ion exchanges through respiratory and chloride cells in freshwater and seawater adapted teleosteans. American Journal of Physiology, 238, R260-268.

Gorbman, A., Dickhoff, W.W., Mighell, J.L., Prentice, E.F. and Waknitz, F.W. (1982) Morphological indices of developmental progress in the parr-smolt coho salmon, *Oncorhynchus kisutch.* Aquculture, 28, 1-19.

Graynoth, E. and Hawke. L.J. (1985) Hatchery rearing of sockeye salmon in New Zealand. Proceedings of the Salmon Symposium. Fisheries Research Divison Occasional Publication No. 47, 36-45.

Hardy, C.H. (1983) Origin of New Zealand's sockeye salmon. Freshwater Catch, 18, 11-13.

Hart, R.K. and Oglesby, G.B. (1979) Toxin induced changes in the gill epithelium of rainbow trout (*Salmo gairdneri*). Scanning Electron Microscopy, III 355-361.

Healey, M.C. (1980) Utilization of the Nanaimo River estuary by juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fisheries Bulletin, 77, 653-668.

Hirano, T. and Utida, S. (1971) Plasma cortisol concentration and the rate of intestinal water absorption in the eel, *Anguilla japonica*. Endocrinology Japonica, 18, 47-52.

Hoar, W.S. (1976) Smolt transformation: evolution, behavior, and physiology. Journal of the Fisheries Research Board of Canada, 33, 1234-1252.

Hoar, W.S. (1988) The physiology of smolting salmonids. In Fish Physiology XI The physiology of developing fish. Part b. (Hoar, W.S. and Randall, D.J. ed.) 275-343.

Hootman, S.R. and Philpott, C.H.W. (1979) Ultrachemical localisation of Na^+, K^+ activated ATPase in chloride cells from the gills of a euryhaline teleost. Anatomy Records, 193, 99-130.

Hootman, S.R. and Philpott, C.W. (1979) Ultracytochemical localisation of Na^+-K^+ -activated ATPase in chloride cells from the gills of a euryhaline teleost. Anatomical Records, 193, 99-130.

Hopkins, C.L. (1981) Juvenile production and yield in quinnat salmon. Proceedings of the Salmon Symposium. Fisheries Research Divison Occasional Publication No 30, 11-14.

Hossler, F.E. (1980) Gill arch of the mullet, *Mugil cephalus* III. Rate of response to salinity change. American Journal of Physiology, 7, R160-R164.

Hossler, F.E., Ruby, J.R. and McIlwain, T.D. (1979) The gill arch of the mullet, *Mugil cephalus* I. Surface ultrastructure. The Journal of Experimental Zoology, 208, 379-398.

Hossler, F.E., Musil, G., Karnaky, K.J. and Epstein, F.H. (1985) Surface ultrastructure of the gill arch of the Killifish *Fundulus heteroclitus*, from seawater and freshwater, with special reference to the morphology of apical crypts of chloride cells. Journal of Morphology, 185, 377-386.

Hwang, P.P and Hirano, R. (1985) Effects of environmental salinity on intercellular organization and junctional structure of chloride cells in early stages of teleost development. The Journal of Experimental Zoology, 236, 115-126.

Hwang, P.P. (1987) Tolerance and ultrastructural responses of branchial chloride cell to salinity changes in the euryhaline teleost *Oreochromis mossambicus*. Marine Biology 94, 643-649.

Hwang, P.P. and Hirano, R. (1985) Effects of environmental salinity on intercellular organiation and junctional structure of chloride cells in early stages of teleost development. The Journal of Experimental Zoology, 236, 115-126.

Idler, D.R. and Truscott, B. (1972) Corticosteroids in fish. In "Steroids in Nonmammalian Vertebrates" (D.R.Idler, ed), 126-252. Academic Press, London/New York.

Jackson, A.J. (1981) Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to sea water. Aquaculture, 24, 143-151.

Johnson, S.L., Ewing, R.D. and Lichatowich, J.A. (1977) Characterization of gill (Na^+-K^+)- ctivated adenosine triphosphatase from chinook salmon, *Oncorhynchus tshawytscha.* Journal of Experimental Zoology, 199, 345-354.

Johnsson, J. and Clarke, W.C. (1988) Development of seawater adaptation in juvenile steelhead trout (*Salmo gardneri*)-effects of size, temperature and photoperiod. Aquaculture, 71, 247-263.

Johnston, C.E. and Cheverie, J.C. (1985) Comparative analysis of ionoregulation in rainbow trout (*Salmo gairdneri*) of different sizes following rapid and slow salinity adaptation. Canadian Journal of Fisheries and Aquatic Sciences, 42, 1994-2003.

Johnston, C.E., (1983) Seasonal changes in gill (Na^+K^+)-ATPase activity in Atlantic salmon retained in fresh water after smolting. Transactions of the American Fisheries Society, 112:720-724.

Karnaky, K.J. (1980) Ion-secreting epithelia: chloride cells in the head region of *Fundulus heteroclitus.* American Journal of Physiology, 238, 185-198.

Karnaky, K.J. (1986) Structure and function of the chloride cell of *Fundulus heteroclitus* and other teleosts. American Zoology, 26, 209-224.

Karnaky,K.J., Ernst, S.A. and Philpott, C.W. (1976) Teleost chloride cell. I. Response of pupfish *Cyprindon variegatus* gill Na^+,K^+-ATPase and chloride cell fine to structure to various high salinity environments The Journal of Cell Biology, 70, 144-156.

Karnaky, K.J., Kinter, L.B., Kinter, W.B. and Stirling, C.E. (1976) Teleost chloride cell. II. Autoradiographic localization of the gill Na^+,K^+-ATPase in killifish *Fundulus heteroclitus* adapted to low and high salinity enviroments. The Journal of Cell Biology, 70, 157-177.

Knutsson, S. and Grav, T. (1976) Seawater adaptation in Atlantic salmon (*Salmo salar L.*) at different experimental temperatures and photoperiods. Aquaculture, 8, 169-187.

Langdon, J.S. and Thorpe, J.E. (1984) Response of the gill Na^+-K^+-ATPase activity succinic dehydrogenase activity and chloride cells to saltwater adaptation in Atlantic salmon, *Salmo salar L.*, parr and smolt. Journal of Fish Biology, 24, 323-331.

Langdon, J.S. and Thorpe, J.E. (1985) The ontogeny of smoltification: developmental patterns of gill Na^+/K^+-ATPase, SDH, and chloride cells in juvenile Atlantic salmon, *Salmo salar* L. Aquaculture, 45, 83-95

Langdon, J.S., Thorpe, J.E. and Roberts, R.J. (1984) Effects of cortisol and ACTH on gill Na^+/K^+-ATPase, SDH and chloride cells in juvenile Atlantic salmon *Salmo salar* l. Comparative Biochemistry and Physiology, Vol 77A, No. 1, 9-12.

Langhorne, P. and Simpson, T.H. (1986) The interrelationship of cortisol, gill (Na^++K^+) ATPase, and homeostasis during the parr-smolt transformation of Atlantic salmon (*Salmo salar* L.) General And Comparative Endocrinology, 61, 203-213.

Laurent, P. (1984) Gill internal morphology. In Fish Physiology, X, Gills, Part A (Editors, Hoar, W.S. and Randall, D.J.) 73-183.

Laurent, P. Dunel, S. (1980) Morphology of gill epithelia in fish. American Journal of Physiology, 238, 147-159.

Leatherland, J.F. (1985) Studies of the correlation between stress-response, osmoregulation and thyroid physiology in rainbow trout, *Salmo gairdnerii*(Richardson). Comparative Biochemistry and Physiology, 80A, 523-531.

Loretz, C.A., Collie, N.L., Richman III, N.H. and Bern, H.A. (1982) Osmoregulatory changes accompanying smoltification in coho salmon. Aquaculture, 28, 67-74.

Maetz, J. (1971) Fish gills: mechanisms of salt transfer in freshwater and seawater. Philosophical Transactions of the Royal Society of London, B 262, 209-249.

Mahnken, C., Prentice, E., Waknitz, W., Monan, G., Sims, C. and Williams. J. (1982) The application of recent smoltification research to public hatchery releases: an assessment of size/time requirements for Columbia River hatchery coho salmon (*Oncorhynchus kisutch*). Aquaculture, 28, 251-268.

Mallatt, J. (1985) Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian Journal of Fisheries and Aquatic Sciences, 42, 630-648.

Markert, J.R and Vanstone, W.E. (1966) Pigments in the belly skin of coho salmon (*Oncorhynchus kisutch*) Journal of the Fisheries Research Board of Canada, 23, 1095-1098.

Marshall, W.S. (1978) On the involvement of mucous secretion in teleost osmoregulation. Canadian Journal of Zoology, 56, 1008-1091.

Matthews, G.M., Park, D.L., Achord, S. and Ruehle, T.E. (1986) Static seawater challenge test to measure relative stress levels in spring chinook salmon smolts. Transactions of the American Fisheries Society, 115, 236-244.

Mazeaud, M.M. and Mazeaud, F. (1981) Adrenergic response to stress in fish. In Stress and Fish (A.D. Pickering, ed.) London and New York: Academic Press 49-75.

Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M. (1977) Primary and secondary effects of stress in fish: some new data with a general review. Transactions of the American Fisheries Society, 106, 203-211.

McCormick, S.D. and Saunders, R.L. (1987) Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth and metabolism. In Commom Strategies of Anadromous and Catadromous Fishes (Dadswell et al. ed.) American Fisheries Society Symposium 1, 561pp.

McCormick, S.D. and Saunders, R.L. (1987) Physiological adaptions to marine life. American Fisheries Society, 1, 211-229.

Miyamoto, T., Machida, Y. and Kawashima, S. (1986) Influence of environmental salinity on the development of chloride cells of freshwater and brackish-water medaka, *Oryzias latipes*. Zoological Science, 3, 859-865.

Nichols, D.J. and Weisbart, M. (1985) Cortisol dynamics during seawater adaptation of Atlantic salmon *Salmo salar*. American Journal of Physiology, 248, 651-659.

Nishioka, R.S., Bern, H.A., Lasi, K.V., Nagahama, Y. and Grau, E.G. (1982) Changes in the endocrine organs of coho salmon during normal and abnormal smoltification -an electron - microscope study. Aquaculture, 28, 21-38

Odell, W.D. (1980) Use of charcoal to separate antibody complexes from free ligand in radioimmunoassay. Methods in Enzymology, 70, 274-280.

Ogasawara, T. and Hirano, T. (1984) Changes in osmotic water permeability of the eel gills during seawater adaptation. Journal of Comparative Physiology, B, 154, 3-11.

Ogawa, M. (1974) The effects of bovine prolactin, sea water and environmental calcium on water influx in isolated gills of the euryhaline teleosts *Anguilla japonica* and *Salmo gairdneri*. Comparative Biochemistry and Physiology, 49A, 545-554.

Ogawa, M. (1975) The effects of prolactin, cortisol and calcium-free environment on water influx in isolated gills of Japanese eel, *Anguilla japanica*. Comparative Biochemistry and Physiology, 52A, 539-543.

Oikari, A. and Soivio, A. (1975) Influence of sampling methods and anaesthetization on various haematological parameters of several teleosts. Aquaculture, 6, 171-180.

Olivereau, M., (1962) Modification de l'interenal du smolt (*Salmo salar* L.) au cours du passage d'eau douce en eau de mer. General and Comparative Endocrinology, 2, 565-573.

Patino, R., Redding, J.M. and Schreck, C.B. (1987) Interrenal secretion of Corticosteroids and plasma cortisol and cortisone concentrations after acute stress and during seawater acclimation in juvenile coho salmon (*Oncorhynchus kisutch*). General and Comparative Endocrinology, 68, 431-439.

Patino, R., Schreck, C.B. and Redding, J.M. (1985) Clearance of plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Journal of Comparative Biochemistry and Physiology, 82A, 531-535.

Patino, R., Schreck, C.B., Banks, J.L. and Zaugg, W.S. (1987) Effects of rearing conditions on the developmental physiology of smolting coho salmon Transactions of the Ameican Fisheries Society, 116, 234-247.

Pereira, D.L. and Adelman I,R. (1985) Interactions of temperature, size and photoperiod on growth and smoltification of chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture, 46, 185-192.

Peterson, G.L.A. (1978) Simplified method for analysis of inorganic phosphate inthe presence of interfering substances. Analytical Biochemistry, 84, 164-172.

Philpott, C.W. (1980) Tubular system membranes of teleost chloride cells: osmotic response and transport sites. American Journal of Physiology, 238, 171-184.

Pic, P., Mayer-Gostan, N. and Maetz, J. (1974) Branchial effects of epinephrine in the seawater-adapted mullet. I. Water permeability. American Journal of Physiology 226, 698-702.

Pic, P., Mayer-Gostan, N. and Maetz, J. (1975) Branchial effects of epinephrine in the seawater adapted mullet. II. Na^+ and Cl^- extrusion. American Journal of Physiology 228, 441-447.

Pickering, A.D. (1981) Stress and Fish. London and New York: Academic Press 367pp.

Pickering, A.D. and Pottinger, .G. (1983) Seasonal and diel changes in plasma cortisol levels of the brown trout, *Salmo trutta* L. General and Comparative Endocrinology, 49, 232-239.

Pickering, A.D. and Pottinger, T.G. (1985) Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. Journal of Fish Biology, 27, 611-619.

Pickering, A.D., Pottinger, T.G. and Christie, P. (1982) Recovery of the brown trout, *Salmo trutta* L., from acute handling stress; a time-course study. Journal Fish Biology, 20, 229-244.

Pickering, A.D., Pottinger, T.G. and Sumpter, J.P. (1986) Independence of the pituitary-interrenal axis and melanotroph activity in the brown trout, *Salmo trutta* L., under conditions of environmental stress. General and Comparative Endocrinology, 64, 206-211.

Pisam, M (1981) Membrannous systems in the "chloride cell" of teleostean fish gill; their modification in response to the salinity of the environment. The Anatomical Record, 200, 401-414.

Pisam, M., Caroff, A. and Ranbourg, A. (1987) Two types of chloride cells in the gill epithelium of a freshwater - adapted euryhaline fish: *Lebistes reticulatus*; their modifications during adaptation to saltwater. The American Journal of Anatomy, 179, 40-50.

Primmett, D.R.N., Eddy, F.B., Miles, M.S., Talbot, C. and Thorpe, J.E. (1988) Transepithelial ion exchange in smolting Atlantic salmon (*Salmo salar* L.). Fish Physiology and Biochemistry, 5, 181-186.

Prosser, C.L. (1973) Comparative animal physiology. 3rd edition Philadelphia: W.B. Saunders. 457pp.

Redding, J.M. and Schreck, C.B. (1983) Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. Transactions of the American Fisheries Society, 112, 800-807.

Redding, J.M., Schreck, C.B., Birks, E.K. and Ewing, R.D. (1984) Cortisol and its effects on plasma thyroid hormone and electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology, 56, 146-155.

Richman, N.H. and Zaugg, W.S. (1987) Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). General and Comparative Endocrinology, 65, 189-198.

Richman, N.H. III. and Zaugg, W.S. (1987) Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon (*Oncorhynchus kisutch*). General and Comparative Endocrinology, 65, 189-198.

Ricker, W.E. (1940) On the origin of kokanee, a freshwater type of sockeye salmon. Transactions of the Royal Society of Canada 34, 121-135.

Robertson, L., Thomas, P., Arnold, C.R. and Trant, J.M. (1987) Plasma cortisol and secondary stress responses of red drum to handling, transport, rearing density, and a disease outbreak. The Progressive Fish-Culturist, 49, 1-12.

Sandor, T. (1979) Comparative aspects of steroid biosynthesis in fish. Proceedings of the Indian National Science Academy. B. 45, 391-406.

Sardet, C., Pisam, M. and Maetz, J. (1979) The surface epithelium of teleostean fish gills. Cellular and Junctional adaptations of the chloride cell in relation to salt adaptation. Journal of Cell Biology, 80, 96-117.

Sargent, J.R., Bell, M.V. and Kelly, K.F. (1980) The nature and properties of sodium ion plus potassium ion-activated adenosine triphosphatase and its role in marine salt secreting epithelia. In Epithelial Transport in the lower Vertebrates (B. Lahlou ed.) Cambridge University Press, London and New York, 251-267.

Sargent, J.R., Thomson, A.J. and Bornancin, M. (1975) Activities and localisation of succinic dehydrogenase and N^+/K^+ - activated adenosine triphosphatase in the gills of eels (*Anguilla anguilla*) adapted to freshwater and sea water. Comparative Biochemistry and Physiology, 51B, 75-79.

Schreck, C.B. (1981) Stress and compensation in teleostean fishes: response to social and physical factors. In Stress and Fish (A.D. Pickering, ed.) London and New York: Acadamic Press 295-321.

Schreck, C.B. and Lorz, A.W. (1978) Stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. Journal of the Fisheries Research Board of Canada, 35, 1124-1129.

Schwalme, K. and MacKay, W.C. (1985) The influence of exercise-handling stress on blood lactate, acid-base and plasma glucose status of northern pike (*Esox lucius* L.). Canadian Journal of Zoology, 63, 1125-1129.

Selye, H. (1956) The Stress of Life. New York, Toronto, London: McGraw-Hill Book Co., nc.

Seyle, H. (1950) Stress and the general adaptation syndrome. British Medical Journal 1, 1383-1392.

Sheridan, M.A., Allen, W.V. and Kerstetter, T.H. (1985A) Changes in the fatty acid composition of steelhead trout, *Salmo gairdneri* Richardson, associated with parr-smolt transformation. Comparative Biochemistry and Physiology, 80B, 671-676.

Sheridan, M.A., Allen, W.V. and Kerstetter, T.H. (1983) Seasonal variations in the lipid composition of the steelhead trout, *Salmo gairdneri* Richardson, associated with the parr-smolt transformation. Journal of Fish Biology, 23, 125-134.

Sheridan, M.A., Allen, W.V. and Kerstetter, T.H. (1985) Changes in the fatty acid composition of steelhead trout, *Salmo gairdnerii* Richardson, associated with parr-smolt transformation. Journal of Comparative Biochemistry and Physiology, 80B, 671-676.

Shirai, N. and Utida, S. (1970) Development and degeneration of the chloride cell during seawater and freshwater adaptation of the Japanese eeel, *Anguilla japonica*. Z. Zellforsch., 103, 247-264.

Smart, G.R. (1981) Aspects of water quality producing stress in intensive fish culture. In Stress and Fish (A.D. Pickering, ed.) London and New York: Academic Press, 277-293.

Soivio, A. and Nikinmaa, M. (1981) The swelling of erthrocytes in relation to oxygen affinity of the blood of the rainbow trout, *Salmo gairdneri* Richardson. In Stress and Fish, (Pickering ed.) 103-119.

Soivio, A., Virtanen, E. and Muona, M. (1988) Desmoltification of heat-accelerated Baltic salmon (*Salmo salar*) in brackish water. Aquaculture, 71, 89-97.

Specker, J.L. and Richman, N.H., III (1984) Environmental salinity and the thyroidal response to thyrotropin in juvenile coho salmon (*Oncorhynchus kisutch*). Journal of Experimental Zoology, 230, 329-333.

Specker, J.L. and Schreck, C.B. (1980) Stress responses to transportation and fitness for marine survival in coho salmon (*Oncorhynchus kisutch*) smolts. Canadian Journal of Fish and Aquatic Sciences, 37, 765-769.

Specker, J.L. and Schreck, C.B. (1982) Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Journal of Comparative Biochemistry and Physiology, 78A , 441-444.

Stewart, L. (1980) A history of migratory salmon acclimation experiments in parts of the Southern Hemisphere and the possible effects of oceanic currents and gyres upon their outcome. Advances in Marine Biology, 17, 397-466.

Strange, R.J. and Schreck, C.B. (1978) Anaesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*). Journal of the Fisheries Research Board of Canada, 35, 345-349.

Strange, R.J., Schreck, C.B. and Golden, J.T. (1977) Corticoid stress response to handling and temperature in salmonids. Transactions of the American Fisheries Society, 106, 213-218.

Sumpter, J.P., Dye, H.M. and Benefey, T.J. (1986) The effects of stress on plasma ACTH -MSH, and cortisol levels in salmonid fishes. General and Comparative Endocrinology, 62, 377-385.

Sweeting, R.M. and McKeown, B.A. (1987) Growth Hormone and seawater adaptation in coho salmon, *Oncorhynchus kisutch*. Journal of Comparative Biochemistry and Physiology, 88A, 147-151

Sweeting, R.M., Wagner, G.F. and McKeown, B.A. (1985) Changes in plasma glucose, amino acid nitrogen and growth hormone during smoltification and seawater adaptation in coho salmon, *Oncorhynchus kisutch*. Aquaculture, 45, 185-197.

Thomson, A.J. and Sargent, J.R. (1977) Changes in the levels of chloride cells and (Na^+ - K^+) dependent ATPase in the gills of yellow and silver eels adapting to seawater. Journal of Experimental Zoology, 200, 33-40.

Thorpe, J.E., Bern, H.A., Saunders, R.L. and Soivio, A. (eds.) (1985) Salmonid Smoltification II. Aquaculture, 45, 403pp.

Towle, D.W. (1981) Role of Na^+ + K^+-ATPase in ionic regulation by marine and estuarine animals. Marine Biology Letters 2, 107-122.

Unwin, M.J. (1981) Aspects of juvenile quinnat salmon outmigration from Glenariffe stream. Proceedings of the Salmon Symposium. Fisheries Research Divison Occasional Publication No 30, 15-20.

Virtanen, E. and Soivio, A. (1985) The patterns of T_3, T_4, cortisol and Na^+-K^+-ATPase during smoltification of hatchery-reared *Salmo salar* and comparison with wild smolts. Aquaculture, 45, 97-109.

Wagner, H.H., Conte, F.P. and Fessler J.L. (1969) Development of osmotic and ionic regulation in two races of chinook salmon *Oncorhynchus tshawytscha*. Comparative Biochemistry and Physiology, 29, 325-341.

Wedemeyer, G.A. and McLeay, D.J. (1981) Methods for determining the tolerances of fishes to environmental stressors. In Stress and Fish (A.D. Pickering, ed.) London and New York: Academic Press 247-275.

Wedemeyer, G.A. and Yasutake, W.T. (1977) Clinical methods for the assessment of the effects of environmental stress on fish health. United States Fish and Wildlife Service Technical Paper 89, 18pp.

Wedemeyer, G.A., Saunders, R.L. and Clarke, W.C. (1980) Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Marine Fisheries Review, 42, 1-14.

Weisbart, M. and McGowan, L. (1984) Radioimmunoassay of cortisone in the adult Atlantic salmon *Salmo salar L.* General and Comparative Endocrinology, 55, 429-436.

Wells, R.M.G., Tetens, V. and DeVeries, A.L. (1984) Recovery from stress following captue and anaesthesia of antarctic fish: haematology and blood chemistry. Journal of Fish Biology, 25, 567-576.

White, A. and Fletcher, T.C. (1986) Serum cortisol, glucose and lipids in plaice (*Pleuronectes platessa L.*) exposed to starvation and aquarium stress. Comparative Biochemistry and Physiology, 84A, 649-653.

Young, G. (1986) Cortisol secretion *in vitro* by the interrenal of coho salmon (*Oncorhynchus kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol. General and Comparative Endocrinology, 63, 191-200.

Zaccone,G. (1981) Effect of osmotic stress on the chloride and mucous cells in the gill epithelium of the fresh-water teleost *Barbus filamentosus* (Cypriniformes, Pisces). A structural and histochemical study. Acta histochem, 68, 147-159.

Zaugg, W.S. (1981) Advanced photoperiod and water temperature effects on gill Na^+-K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences, 38, 758-764.

Zaugg, W.S. (1981) Advanced photoperiod and water temperature on gill Na^+-K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences, 38, 758-764.

Zaugg, W.S. (1982) Some changes in smoltification and seawater adaptability of salmonids resulting from environmental and other factors. Aquaculture, 28, 143-151.

Zaugg, W.S. and McLain, L.R. (1970) Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon, *Oncorhynchus kisutch.* Comparative Biochemistry and Physiology, 35, 587-596.

Zaugg, W.S. and McLain, L.R. (1970) Gill sampling as a method of following biochemical changes: ATPase activities altered by ouabain injection and salt water adaption. Comparative Biochemistry and Physiology, 38B, 501-506.

Zaugg, W.S. and McLain, L.R. (1972) Changes in the gill adenosinetriphosphatase activity associated with parr-smolt transformation in steelhead trout, coho, and spring chinook salmon. Journal of the Fisheries Research Board of Canada, 29, 167-171.

Appendix.

Gill Na^+-K^+-ATPase Activity - Chemical Mixtures.

Homogenising Medium.

pH = 7.2

10mM	Na_2EDTA
300mM	sucrose
10mM	2-mercaptoethanol
100mM	Imidazole

Incubation Mediums.

pH = 7.2

A. Total ATPase Activity.

150mM	NaCl
75mM	KCl
20mM	$MgCl_2$
100mM	Imidazole
10mM	Na_2ATP

B. Mg^{2+}-ATPase.

150mM	NaCl
75mM	KCl
20mM	$MgCl_2$
100mM	Imidazole
10mM	Na_2ATP
0.58mM	Ouabain